SUBUNITS OF LIMNODRILUS ERYTHROCRUORIN

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SUMMARY

The dissociation of the erythrocruorin of the oligochaete Limnodrilus gotoi was investigated using polyacrylamide gel electrophoresis at neutral pH. In the presence of 0.1% SDS, the erythrocruorin dissociated into five subunits possessing molecular weights of 13,000 (1), 20,000 (2), 23,000 (3) 25,000 (4) and 47,000 (5). In the presence of SDS and mercaptoethanol, the erythrocruorin dissociated into two subunits, whose molecular weights were 13,000 (I) and 28,000 (II). Subunit I accounts for 70-80% of the whole molecule. SDS electrophoresis of the isolated subunits 1 through 5 in the presence of mercaptoethanol showed that subunit I was derived from both subunits 1 and 5, while subunit II was derived from subunits 2-4. These results suggest that Limnodrilus erythrocruorin consists of at least five polypeptide chains: two chains of 13,000 and three chains of 28,000.

INTRODUCTION

Annelid erythrocruorins share several properties: a sedimentation constant of 50 to 60S, a molecular weight of about 3×10^6 and an acidic isoelectric point (Svedberg and Eriksson-Quensel, 1933). In electron microphotographs these extracellular molecules appear to consist of two superimposed hexagonal arrays of twelve spherical subunits possessing a diameter of 220-260A and a height of 105-175A (Levin, 1963; Roche, 1965).

The erythrocruorin of the fresh water oligochaete Limnodrilus gotoi possesses a molecular weight of 3.01×10^6, a diameter of 220A and a height of 160A (Yamagishi et al., 1966). It contains 104 ± 5 free N-terminal groups consisting of valyl, glutamyl, threonyl and seryl residues in molar ratios of 4:3:1:1. A model of its quaternary structure put forward by Yamagishi et al. (1966) proposed that each of the twelve principal subunits consisted of nine smaller subunits corresponding to four different polypeptide chains of 28,000 occurring in molar ratios of 4:3:1:1.

We report below the results of a determination of the molecular weights of the constituent polypeptide chains of the erythrocruorin of Limnodrilus gotoi.

MATERIALS AND METHODS

The erythrocruorin was prepared from live Limnodrilus gotoi using the method described by Yamagishi et al., (1966). Electrophoresis in 10% acrylamide gels in the presence of 0.1% (w/v) sodium dodecyl sulfate (SDS) was performed according to the method of Weber et al. (1972) at pH 7.2 in 0.05 M sodium phosphate buffer. All proteins were incubated with and without 1% (w/v) mercaptoethanol at 45° C for 1 hr. prior to electrophoresis. About 15 ug of protein was applied to each gel. The electrophoresis was carried out at 25° C, at a constant current of 8 ma per gel and employing Bromphenol Blue as a tracking dye. The following proteins were used as molecular weight standards: bovine serum albumin (68,000), γ-globulin H chain (50,000), ovalbumin (43,000), lactate dehydrogenase (36,000), pepsin (35,000), carbonic anhydrase (29,000), chymotrypsinogen A (25,700), γ-globulin L-chain (23,500), β-lactoglobulin (18,300), myoglobin (17,200) and cytochrome c (11,700). The gels were

stained overnight with Coomassie Brilliant Blue R 250 and destained by diffusion in 25% (v/v) methanol and 7.5 (v/v) acetic acid solution. The stained gels were scanned on a Cary model 15 spectrophotometer at 555 nm at a fixed slit width of 0.05 mm and at rates of 5 cm per hour. The areas under the peaks were determined by weighing the tracings of the curves.

Limnodrilus erythrocruorin subunits were obtained by SDS electrophoresis in the absence of mercaptoethanol using loads of approximately 30 to 50 ug of protein per gel. One of the gels was stained for 15 min. and then destained to indicate the positions of the observed bands. Gel slices corresponding to each band from the remaining unstained gels were cut out and the gel slices placed on top of freshly prepared gels. The gel slices were incubated in situ with 100 to 200 ul of 0.05 M sodium phosphate buffer pH 7.2 containing 1% SDS with and without 5% (w/v) mercaptoethanol for 2 hrs. The gels were then electrophoresed in 0.1% SDS.

RESULTS AND DISCUSSION

The SDS electrophoretic patterns of Limnodrilus gotoi erythrocruorin obtained in the absence and presence of mercaptoethanol are shown in Fig. 1,A and B, respectively. In the presence of SDS only the erythrocruorin dissociated into five subunits. Their molecular weights calculated from the plot of log (molecular weight) versus mobility obtained with the standard proteins are given in Table 1. In the presence of SDS and mercaptoethanol the erythrocruorin dissociated into two subunits (Fig. 1,B; Table 1).

The results of the reelectrophoresis of the fractions corresponding to subunits 1, subunits 2-4 and subunit 5 obtained by SDS electrophoresis in the absence of mercaptoethanol are also

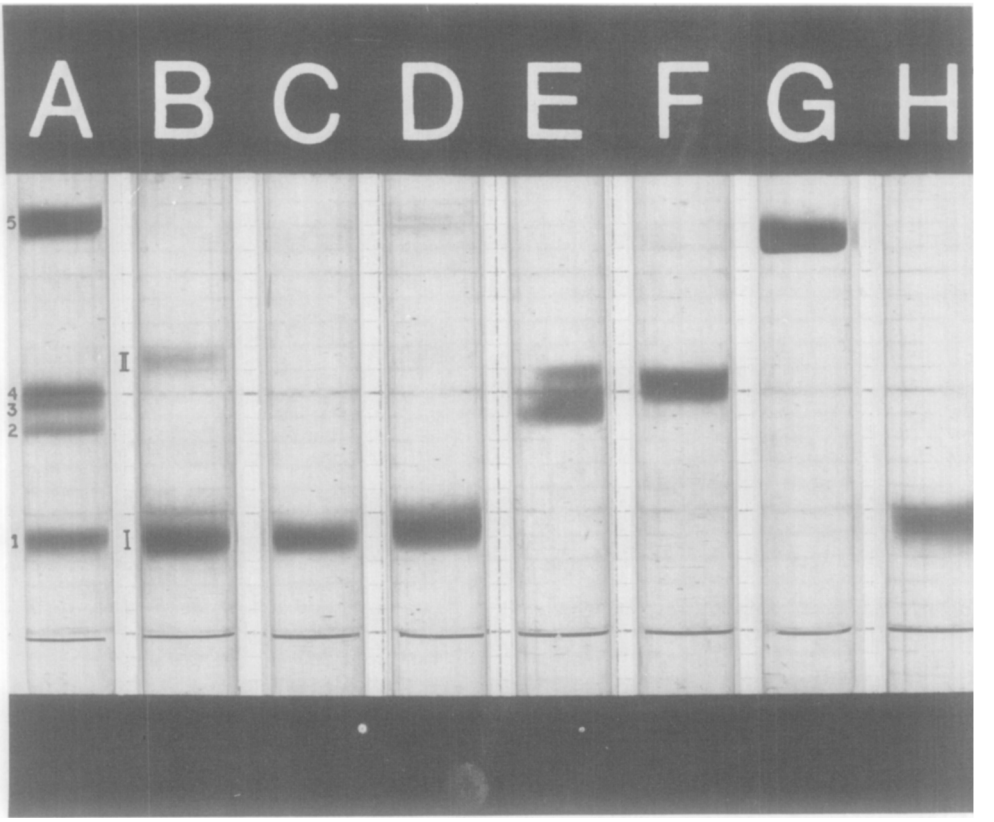

Figure 1. The results of SDS electrophoresis of Limnodrilus erythrocruorin (A, without and B, with mercaptoethanol) and of its subunits isolated by SDS electrophoresis in the absence of mercaptoethanol: subunit 1 (C,D), subunits 2-4 (E,F) and subunit 5 (G,H); (C,E,G - without mercaptoethanol; D,F,H - with mercaptoethanol). The gels were aligned with respect to the position of the tracking dye.

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shown in Fig. 1. Subunits 1 and 5 (Fig. 1,C,G) produced in mercaptoethanol subunit I (Fig. 1,D,H). The fraction corresponding to subunits 2,3 and 4 (Fig. 1,E) when reelectrophoresed in mercaptoethanol, produced subunit II (Fig. 1,F).

The pattern of dissociation of Limnodrilus gotoi erythro-

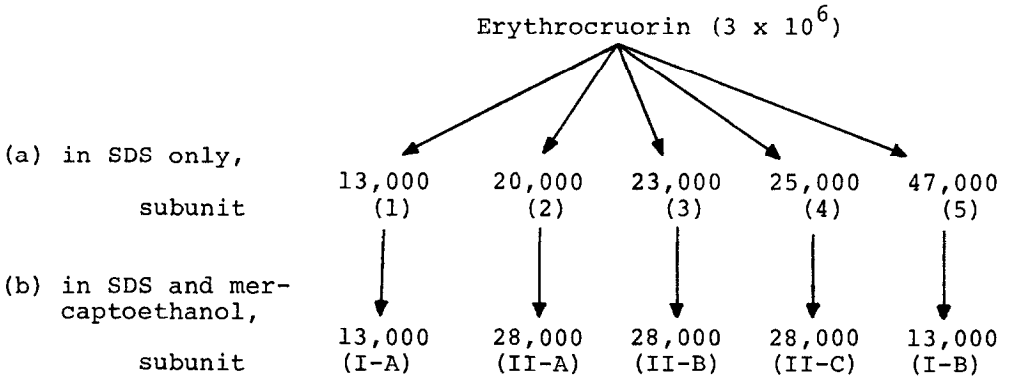
Table 1

MOLECULAR WEIGHTS AND RELATIVE PROPORTIONS OF LIMNODRILUS ERYTHROCRUORIN SUBUNITS OBTAINED BY SDS ELECTROPHORESIS

Band No.	Without mercaptoethanol		Band No.	With mercaptoethanol	
	Mol. wt.[a]	Area[a]		Mol. wt.[a]	Area[a]
1	13,000±1,500	27±2	I	13,000±2,000	80±3
2	20,000±1,000		II	28,000±1,000	20±3
3	23,000±1,000	31±2			
4	25,000±1,000				
5	47,000±1,600	42±3			

[a]Averages of three determinations.

cruorin observed by SDS electrophoresis can be summarized as follows:



The finding that both subunits 1 and 5 produced in mercaptoethanol subunits possessing the same molecular weight suggests that subunits 1 and 5 correspond to two different polypeptide chains (I-A and I-B, respectively), since in SDS only one of them (I-B) aggregates to what is propably a tetramer (subunit 5)

while the other one does not. SDS electrophoretic determination of molecular weights is unreliable for polypeptide chains of 15,000 and less (Weber et al., 1972); hence, 13,000 is probably a lower limit for the molecular weight of subunits I-A and I-B. It is evident from the densitometric results that the chains I-A and I-B comprise 70-80% of the Limnodrilus erythrocruorin (Table I).

The observation that in the presence of SDS the molecular weights of subunits 2,3 and 4 are all different and less than the molecular weight of subunit II in SDS and mercaptoethanol, can be explained by assuming that there are different arrangements of intramolecular disulfide bonding in subunits 2,3 and 4. It is known that unreduced proteins containing disulfide bonds do not in general adopt as extended a hydrodynamic shape in the presence of SDS as do the reduced proteins (Fish et al., 1970). The presence of residual structures will cause the three subunits to assume different hydrodynamic shapes and hence different mobility in SDS. In SDS and mercaptoethanol they all assume a completely random coil shape and exhibit the same mobility characteristic of their true molecular weight. Although subunits 2,3 and 4 all produce subunit II in mercaptoethanol, they correspond probably to different polypeptide chains (II-A, II-B and II-C) possessing different intramolecular disulfide bonding arrangements. Thus, Limnodrilus erythrocruorin consists of at least five polypeptide chains: I-A and I-B possessing a molecular weight of ca. 13,000, and II-A,B, C, each having a molecular weight of about 28,000.

The minimum molecular weight of 28,000 found on the basis of N-terminal and heme determinations (Yamagishi et al., 1966) suggests that one heme group occurs per dimer of either or both chains I-A and I-B. A similar arrangement has been postulated in

the case of Arenicola erythrocruorin (Waxman, 1971) and is also likely in Pista erythrocruorin (Terwilliger et al., 1975). The molecular weights of chains I-A and I-B clearly indicate that the total number of free N-terminal residues per molecular weight of 3×10^6 should be about double that determined by Yamagishi et al. (1966). It is possible that one of the two chains possesses a blocked N-terminal group. N-terminal determinations of the polypeptide chains of Limnodrilus erythrocruorin separated by SDS electrophoresis, currently in progress, should clarify this point.

Tubifex tubifex, an oligochaete belonging to the same family as Limnodrilus gotoi, possesses an erythrocruorin whose properties have been characterized (Scheler and Schneiderat, 1959; Nakajima and Braunitzer, 1967; Russell and Osborn, 1968) and whose quaternary structure has been found to fit the model proposed for the Limnodrilus molecule (Stockel et al., 1973). The SDS electrophoretic patterns of Tubifex erythrocurorin are very similar to those of Limnodrilus erythrocruorin. It consists of at least four polypeptide chains: two chains of 13,000 accounting for about 80% of the molecule and two chains of 26,000 (Vinogradov et al., manuscript in preparation).

The number and molecular weights of the constituent polypeptide chains of annelid erythrocruorins vary a great deal. In comparison with the Tubifex and Limnodrilus erythrocruorins, that of Lumbricus terrestris, another oligochaete, is known to consist of at least six polypeptide chains (13,000, 14,000, 16,000, 19,000, 31,000 and 37,000) (Shlom and Vinogradov, 1973). Achaete (leech) erythrocruorins consist of at least four to five polypeptides chains with molecular weights between 13,000 and 30,000 (Andonian and Vinogradov, 1975; Andonian et al., 1975). In contrast, polychaete erythrocruorins appear to be simpler than the

oligochaete and achaete molecules. Arenicola erythrocruorin consists of two polypeptide chains, 13,000 and 14,000 (Waxman, 1971); Pista erythrocruorin consists of two subunits possessing molecular weights of 15,500 and 30,000 (Terwilliger et al., 1975), while Cirraformia erythrocruorin consists probably of three subunits with molecular weights in the range 12,000-16,000 (Vinogradov et al., 1975). The very appreciable variation in the number and molecular weights of the constituent polypeptide chains of all the erythrocruorins studied so far stands in contrast to the apparent similarity of the gross quaternary structures of these giant molecules as seen by electron microscopy.

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