CRYSTALLIZATION OF A PROTEIN - PROTEIN COMPLEX : HEMOGLOBIN - HAPTOGLOBIN

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The crystalline state of proteins is very suitable for the elucidation of the molecular arrangement of subunits, by the means of electron microscopy or X ray diffraction patterns. Hemoglobin-Haptoglobin binding leads to the formation of a protein-protein complex (Hb-Hp) of high stability (Jayle 1940) which can be studied by Mossbauer spectrometry (1958) in addition to the above mentionned techniques.

Heme proteins in crystalline state are in sufficiently high concentration to permit Mossbauer spectrometry at high resolution (Lang and Marshall 1966).

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A method of crystallization of Hb-Hp and the characterization of these crystals are described in this report.

Material and methods - Pure human Haptoglobins of genetic type 1-1 and 2-2 were prepared as previously described (Waks and Alfsen 1966 a). Rat Hemoglobin was prepared according to Rosa (1959) and used in the monocarboxy liganded form. The concentration was measured at 540 mμ after converting Hemoglobin in the cyanmet derivative. (Cameron 1965). Some of Hb-Hp crystals were washed by a 3.5 M phosphate buffer (Roche et al 1941).

Microcrystals were studied by phase contrast polarizing and Soret absorption microscopy and their features compared with these of microcrystals of Rat Hemoglobin. Microphotography was carried out by means of Zeiss photomicroscope adapted for polarization microscopy.

Polyacrylamide gel electrophoreses were carried out as described by Raymond and Wang (1960). Amino-acid analyses were performed with a Technicon Auto-Analyser, using a micro method described by Robin et al (1967).

Results - Crystals of Hb-Hp were first observed in Münich during vacuum dialysis against distilled water at 4°, performed in order to study the Mossbauer effect on the concentrated Hb-Hp ; in this case Rat Hemoglobin was Fe^{57} enriched. Crystallization has been reproduced in Paris under similar conditions, using a 10 % Rat Hemoglobin solution which had not been enriched with the heavy isotope. At high concentration Rat Hemoglobin crystallizes spontaneously at 4°. These crystals were then dissolved at pH 9.5 by addition of 1 N KOH. Haptoglobins of genetictype 1-1 and 2-2 were alternatively used at a concentration between 4 and 8 %, at pH 5.0. Equimolar amounts of Haptoglobin and Hemoglobin were calculated using a molecular weight of 85.000 for Hp 1-1 and Hp 2-2 (Waks and Alfsen 1966 b). The molecular weight of Rat Hemoglobin was assumed to be identical to that of Horse Hemoglobin, i.e : 64.500 (Perutz 1965).

Table I. Differential Features of Microcrystals

	Microcrystal form	Soret Absorption	Birefringence	Dichroism and angle of extinction
Rat Hemoglobin	Irregular-Single hexagonal plates, elongated rods and needles Size : 1- 10 μ	Strong	Medium	Mauve - light Green - 43°
Hp 1-1 ° Hb Rat Complex	Irregular - Single Polygonal plates Size : 1- 10 μ	Weak	High	Blue - white - 54°30'
Hp 2-2 ° Hb Rat Complex	Irregular-polygonal plates rare stellates complexes and grains Size : 1-20 μ	Moderate	Low	Blue - white - 53°30'

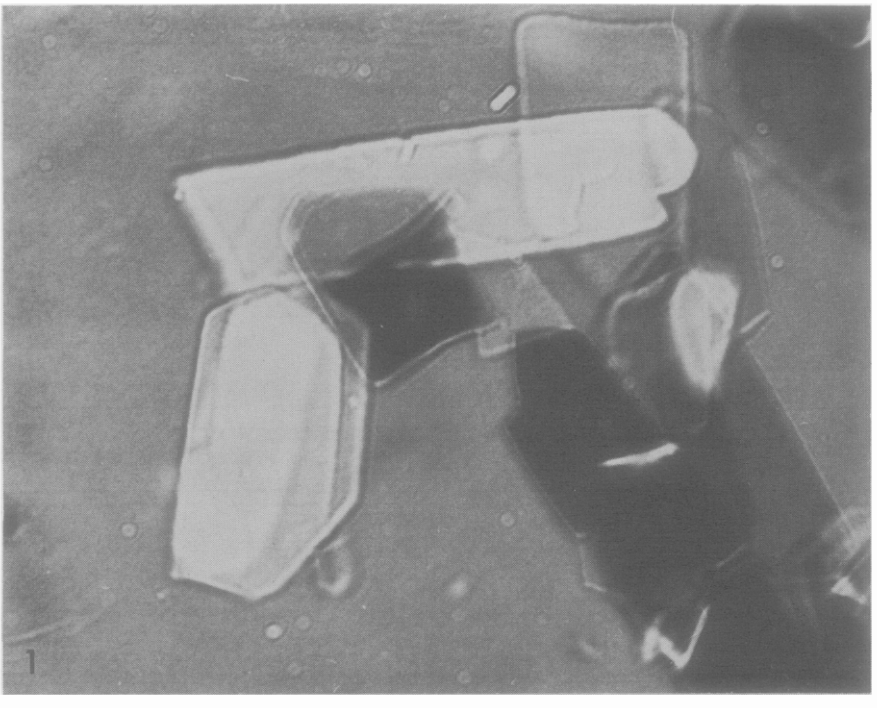

Figure 1 - Polarization microphotograph (X 1100) of crystal of Haptoglogin 1-1. Hemoglobin rat complex; showing irregular dichroic birefringent polygonal plates.

The solutions were mixed and stored over-night at 4°; crystallization occured during that period.

The crystals were washed several times with the 3.5. M phosphate buffer, then dissolved in distilled water. Polyacrylamide gel electrophoreses were performed on a sample of a Rat Hemoglobin solution and compared with the solution of Hb-Hp. Rat Hemoglobin displays several bands of different intensities (Rosa 1959), from the cathode to the anode, Hb-Hp migrates as a single band. At the same time, electrophoreses of dissolved crystals were carried out and compared to the pattern of Rat Hemoglobin solution. As shown in fig.2, the electrophoretic pattern of dissolved crystals consists of a single band. Its migration is identical to that of a Hb-Hp solution.

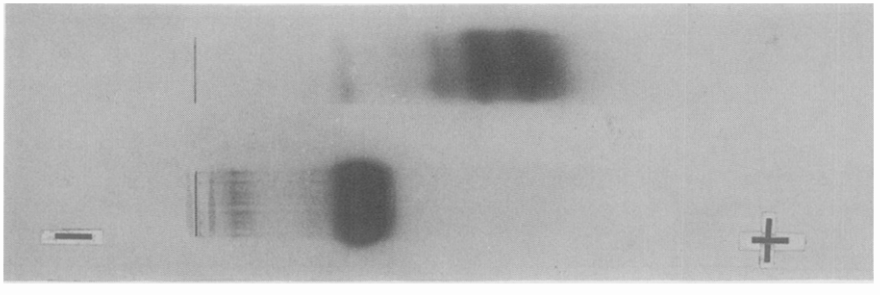

Fig.2 : Polyacrylamide gel electrophoresis patterns at pH 8.9. Upper pattern : Rat Hemoglobin ; lower pattern : dissolved crystals of Hb-Hp. The gel was stained with Amido-Black 10 B.

The crystals obtained after mixing Hemoglobin and Haptoglobin were washed with 3.5. M phosphate, dissolved, dialysed against distilled water and then subunitted to amino acid analysis. The same analysis was performed on Rat Hemoglobin. Preliminary data are summarized in Table II.

TABLE II

Amino-acids	Haptoglogin	Rat Hemoglobin	Crystals
Alanin	3.53	7.70	5.91
Phenylalanin	2.36	6.90	4.00
Histidin	3.12	9.80	4.75

Results are expressed in grams per cent of protein.
The Haptoglobin data are those published by Schultze (1966)

In conclusion, all the results obtained in this work demonstrate that crystals of a protein-protein complex (Hb-Hp) have been obtained and characterized. Stoichiometric studies, electron microscopy and Moessbauer spectra of Hb-Hp in crystalline form are in progress in our laboratories.

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