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Short Communications

Human Spectrin. IV. A Preliminary X-Ray Study

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In an earlier study we found that spectrin plays an important role in controlling the lateral mobility of the human erythrocyte intramembrane particles.[1,2] This can most easily be understood in terms of spectrin being long molecules and forming a molecular meshwork on the cytoplasmic side of the erythrocyte membrane. The reported lengths of spectrin vary from 28 nm to 210 nm.[3,4] We have recently obtained data indicating that spectrin heterodimers are flexible and highly elongated molecules.[5,6] X-Ray fibre analysis has proved to be a very powerful technique for the study of such molecules, and we here report on the first X-ray study of spectrin.

Experimental. Spectrin heterodimers were prepared as described earlier (procedure A)[5] except that recently outdated human blood was used instead of fresh blood. The spectrin heterodimer solutions were dialyzed for 40 h at 0 – 4 °C against 2 × 1 l distilled water pH 7 before the spectrin solution was concentrated. The concentration was either done by ultrafiltration through a Diaflo PM30 membrane or by employing Aquacide II-A. In the latter case the spectrin solutions were placed inside two dialysis bags, one inside the other.

Spectrin films were made by allowing the concentrated spectrin solutions to dry out on sheets of polytetrafluoroethylene. The spectrin films were then cut into 2 – 3 mm wide strips and stretched as described by Atkins and coworkers.[7,8]

The X-ray diffraction photographs were recorded on flat film using a camera with pinhole collimation (0.5 mm diameter) and Ni-filtered Cu$K\alpha$ radiation. The atmosphere in the camera was maintained at the desired relative humidity by placing an appropriate saturated salt solution in the camera. The total pressure in the camera was kept at 20 – 25 Torr (2.6 – 3.3 kPa).

The X-ray diffraction photographs were scanned employing a Joyce MK III CS microdensitometer.

Results and discussion. X-Ray diffraction photographs of strips of unstretched spectrin film exhibited two diffuse concentric rings having maxima at the reciprocal distances 1.0 and 2.2 nm^{-1}, respectively.

Bundles consisting of 6 – 7 spectrin film strips were elongated at 25 °C by loading the bundles with 13 gram and stepwise increasing the relative humidity from 75 to 98 – 100 %. The relative humidity at which the elongation of the bundle of film strips started varied from 88 to 98 % depending on the spectrin concentration procedure used. When the elongation had started it would continue without any further increase of the relative humidity. During the first 1 – 2 h after the onset of the elongation the bundles of spectrin film strips would approximately double their lengths. After that the elongation slowed down and a further increase of the relative humidity to 90 – 100 % was needed to make the

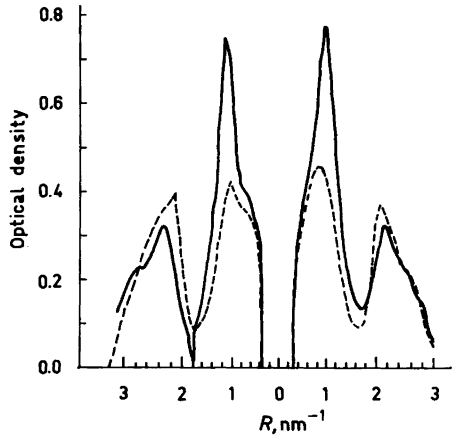

Fig. 1. Densitometer scan of X-ray diffraction photograph of strips of spectrin film elongated at 88 – 95 % relative humidity to 16 times their original lengths. The X-ray diffraction photograph was obtained with the incident X-ray beam at 90° to the long axis of the elongated spectrin fibre and the sample kept at 72 % relative humidity. The X-ray diffraction photograph optical density along the equator (–) and along the meridian (– – –).

bundles elongate to 10–15 times their original lengths. At the end of the elongation the bundle of of spectrin film strips exhibited a fibre-like appearance.

X-Ray diffraction photographs of the elongated spectrin fibres showed two strong reflections on the equator indicating that the elongation of the spectrin fibres resulted in some ordering of the spectrin molecules. The X-ray diffraction photographs of the elongated spectrin fibres are very similar to the X-ray photographs of keratin, myosin and fibrinogen (the kmf-group).[9] A densitomter scan of an X-ray diffraction photograph of an elongated spectrin fibre is shown in Fig. 1. We have not been able to find any spectrin fibre preparation procedure which yields X-ray diffraction photographs containing layer lines.

The observation that X-ray diffraction photographs of elongated spectrin fibres belong to the kmf-group indicates that the spectrin secondary structure to a considerable extent consists of α-helices.[10] This is consistent with circular dichroism measurements of spectrin indicating that spectrin consists of 35–65 % α-helix.[11-14] The ordering resulting from elongation of the spectrin fibres further indicates that the spectrin molecules are highly asymmetric or flexible worm-like/coil-like molecules. This is in agreement with our earlier observations.[5,6,15]

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