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Formation of amyloid-like particles by M131A, M131W, V10F mutant forms of apomyoglobin studied by SAXS technique

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Introduction

Actual topic of the present experiment is the analysis of formation of pathogenic amyloid fibrils causing numerous diseases. This problem is closely related to the protein folding/ misfolding. Recent studies have shown that various proteins, even not related to any known amyloid disease, can aggregate into fibrils under the native fold- destabilizing condition [1] and that normal proteins become toxic in this case. Therefore, recognition of factors that influence protein misfolding is a fundamental problem, whose solution can help in finding effective treatment of amyloid diseases. Intermediate states of the protein can play the role of a "bifurcation point", from which the protein can go to either a "healthy", native structure, or to a "pathological" amyloid structure. Amyloidogenic properties depend on properties of protein chains and specific environments, which can lead to loosening tertiary structure. Apomyoglobin is known to form fibrils under definite conditions. To study this process in detail, we have prepared mutant forms of apomyoglobin M131A, M131W, V10F. Here we present SAXS patterns of these forms.

Experimental

Mutant forms of apomyoglobin (M_w=18 kD) were prepared according to [2]. To obtain amyloid-like particles, the protein solutions were kept at 40^{o}C for 24 hours. The used buffer was 10mM Na-phosphate (pH5.5). Protein concentrations were 5.0 mg/ml. Synchrotron X-ray measurements were done on a small-angle camera BL-15A (Photon Factory, Tsukuba) using CCD-detector. The range of scattering vectors Q=0.008-0.2 Å^{-1} .

Results

It appeared that Guinier plot for all three mutant forms are not linear (not shown) reflecting essential association of protein. Evaluated radii of gyration (Rg) from Guinier plot were 107 Å, 92 Å , 96 Å for M131A, V10F, M131W, respectively. The corresponding values of molecular mass evaluated from I(0) were 496, 400, 256 kD, respectively. The Kratky plot for M131A and V10F demonstrated bell- like shape of scattering patterns (not shown) indicating the compact conformation of protein molecules inside associates. Whereas the maximum on the Kratky plot for M131W is not well expressed as for the above mutants reflecting some disorder of protein molecules inside associates. The essential association of protein molecules permits to elucidate the type of such associates. In Fig.1 cross-section plots of SAXS pattern are presented. One can see the good linear dependence of SAXS patterns reflecting the elongated shape of molecule. The evaluated radii of gyration of cross section (R_c) were 33.4 Å for M131A and V10F, and 30.2 Å for M131W. Calculation of a filament length (L)on the basis of R_g and R_c values gives L= 351 Å for M131A and V10F, and L=316 Å for M131W. Thus, all three mutant forms of apomyoglobin form amyloid-like particles whereas the conformation of protein molecules inside associates is compact for M131A and V10F, and the appropriate conformation for M131W is some disordered.

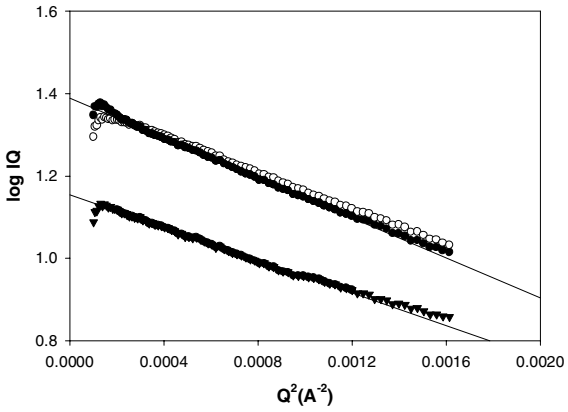

Fig.1 Cross-section plot of SAXS patterns for mutant forms of apomyoglobin. M131A(solid circles), V10F (open circles), M131W (triangles).

References

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