Biological Science

15A/2002G338

Kratky plot as a tool to evaluate the molecular mass of globular proteins

Gennady V. SEMISOTNOV[*1, 3], Alexander A. TIMCHENKO[1, 3], Bogdan S. MELNIK[1], Kazumoto KIMURA[2], Hiroshi KIHARA[3]

[1]Institute of Protein Research., RAS, 142292 Pushchino, Moscow Region, Russia

[2]Division of Medical Informatics., Dokkyo University, School of Medicine, Mibu, Tochigi 321-02, Japan

[3]Department of Physics, Kansai Medical University, Uymahigashi, Hirakata 573-1136, Japan

Introduction

The molecular mass is one of the main characteristics of proteins. The X-ray or neuron scattering allows to determine the molecular mass of proteins by an extrapolation of the scattered intensity to zero scattering angle by Guinier plot [1]. However, this approach requires the knowledge of exact protein concentration, correction on the experimental conditions and absolute scale for the scattered intensity. Besides, it is ineffective in the presence of nonspecific intermolecular association. Here we have proposed the other approach based on Kratky plot maximum position to be dependent on Rg in the case of globular proteins [2]. The small-angle X-ray scattering (SAXS) patterns have been measured for several globular proteins with known molecular weight from 10 kDa up to 800 kDa and the dependence of the Kratky plot maximum position on molecular mass of protein has been determined. This approach permits to evaluate the molecular mass of proteins without the correction on experimental conditions and even in the presence of their moderate nonspecific association.

Materials and methods

The number of globular proteins including horse cytohrome C (10kDa), human α-lactalbumin (14kDa), bovine carbonic anhydrase (29 kDa,) yeast phosphoglycerate kinase (49 kDa) GroES (70kDa), pig muscle lactatdehydrogenase (40 kDa,) and GroEL (800 kDa) have been purified according to published protocols.

The SAXS patterns of the proteins were measured on BL-15A small-angle installation at 23^{0}C. The protein concentration was 5 mg/ml and time of the scattering data accumulation was 300s

Results

Figure 1 shows the dependence of the Kratky plot maximum position on molecular mass for the proteins studied. One can see that the position of the Kratky plot maximum is shifted to smaller values of the scattering vector (S) upon protein molecular mass increase. The incline of regression line is -3.0+/-0.1 which is very close to the expected one for globular proteins.

In contrast to Guinier plot in this case it is not obligatory to know exact protein concentration, correct on the experimental conditions and avoid moderate intermolecular association.

The measurements of the SAXS patterns for the different globular proteins in the presence of their moderate intermolecular association induced by pH and increased ionic strength show that Kratky plot maximum position is less perturbed by the moderate intermolecular association than Guinier plot.

Thus, the use of the Kratky plot to estimate the globular protein molecular mass may be very useful for the study of unknown protein at various experimental conditions especially for structural post genomic studies.

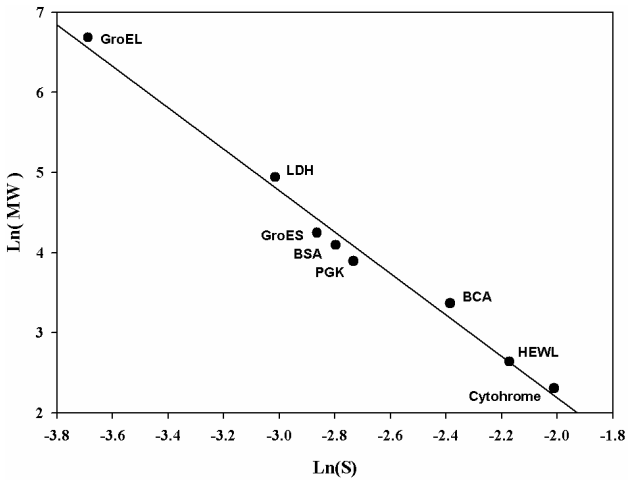

Fig 1

The dependence of Kratky plot maximum position on the molecular mass of globular proteins. The incline is -3.0+/-0.1. The correlation factor is equal to 0.988

References

[1] O Glatter et al., Academic Press, London. 1-515 (1982).

[2] G V Semisotnov et al., J. Mol. Biol., 262, 559 (1996)

* nina@vega.protres.ru