# Conformational changes in arginine kinase upon ligand binding seen by small-angle X-ray scattering

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Small-angle X-ray scattering is used to study the effects of substrate binding to lobster arginine kinase in solution. We measure the radius of gyration of the enzyme in the absence and in the presence of ligands. We find that the radius of gyration decreases by 1.20 ± 0.25 Å upon binding ADP-Mg and L-arginine to form the ternary complex. The same decrease is also observed upon binding ADP-Mg alone or ATP-Mg. These results indicate a large conformational change consistent with the hinge motion of domains observed in other phosphokinases.

*Arginine kinase* *Radius of gyration* *Hinge bending* *X-ray scattering*

## 1. INTRODUCTION

Arginine kinase (EC 2.7.3.3), isolated from lobster (*Homarus vulgaris*) tail muscle, catalyzes the reaction:

$$ADP\text{–}Mg + arginine\text{-}P \rightleftharpoons ATP\text{–}Mg + arginine$$

Like creatine kinase in vertebrates, this guanidino-phosphotransferase plays a major biological role during muscle contraction by supplying ATP to the contractile apparatus. This protein is a monomer with $M_r$ 38000, composed of a single polypeptide chain with 329 residues. The enzyme has been crystallized [1], its amino acid sequence is being determined [2] and its 3-dimensional structure is under study in our laboratory.

Crystallographic analysis has shown that yeast hexokinase is made up of globular domains separated by a cleft in which substrates bind [3,4]. Binding is accompanied by the closing of the cleft through rotation of the domains. Similar mechanisms are assumed for other kinases [5,6]. To test the existence of such a conformational change in arginine kinase, we have measured the radius of gyration of the native enzyme and of its binary and ternary complexes. We observe a 1.20 Å decrease of the radius of gyration in the complexes containing ATP or ADP, which is consistent with a nucleotide-induced hinge rotation of two domains.

## 2. MATERIALS AND METHODS

### 2.1. *Preparation of enzyme*

Arginine kinase is isolated from the tail muscle of *Homarus vulgaris* as in [7]. The enzyme is stored as a suspension in 60% ammonium sulfate (pH 7.0) at 4°C. Protein concentration is determined spectrophotometrically at 280 nm, using an extinction coefficient of 6.7 [8]. The ammonium sulfate precipitate is redissolved and dialysed overnight against 100 mM Tris–HCl, 1 mM dithiothreitol (pH 7.6). When needed, 10 mM ADP (Sigma), 10 mM L-arginine (Merck) or 10 mM ATP (Sigma) are added. These concentrations are about 10-times the respective $K_m$-values.

### 2.2. *Small-angle X-ray scattering experiments*

Experiments are performed using the system in [9]. The X-ray beam from an Elliot GX6 rotating copper anode generator is nickel-filtered, focused by a gold-coated bent glass mirror and collimated by two slits. The sample is held in a quartz



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capillary tube at 20°C. Specimen to detector distance is 436 nm. A vacuum chamber reduces air scattering. Scattered X-ray are recorded on a position-sensitive proportional counter [10]. Intensity data are stored in a multichannel analyser. Background scattering from collimators, air, capillary tube and buffer is measured first, normalized and subtracted from intensities scattered by protein samples. Background corrected scattering intensities are put on an absolute scale [11] and fitted in the Guinier region to a straight line, using a recursive least-squares procedure.

## 3. RESULTS

Small-angle scattering intensities plotted using the Guinier representation are shown in fig.1. Radii of gyration are calculated from the slopes of the curves fitted to straight lines. For each ligand–enzyme complex, the measured radius of gyration is given in fig.2 as a function of protein concentration. Least-squares analyses of unliganded enzyme and ternary complex are shown as smooth lines.

The corresponding values of the radius of gyration, extrapolated to zero protein concentration,

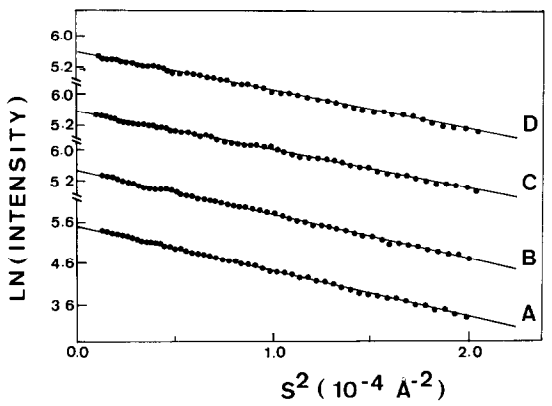


Fig.1. Guinier plots for small-angle X-ray scattering by arginine kinase. Enzyme concentration is 10 mg/ml, Tris–HCl buffer 100 mM (pH 7.6), dithiothreitol 1 mM. Least-squares fits through the experimental data points are shown by lines: (A) unliganded arginine kinase; (B) enzyme in presence of 10 mM L-arginine; (C) enzyme in presence of 10 mM ADP and 20 mM $MgCl_2$; (D) enzyme in presence of 10 mM ADP, 20 mM $MgCl_2$ and 10 mM L-arginine.

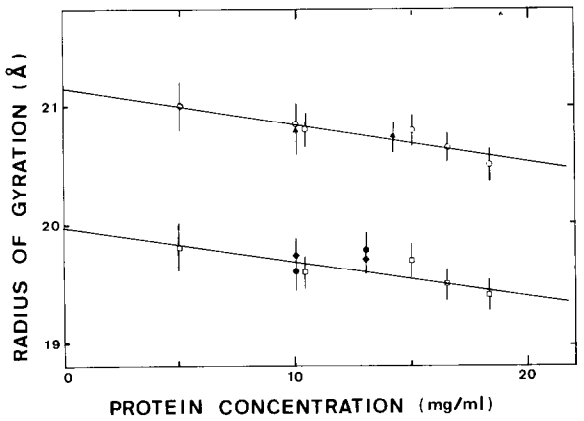


Fig.2. Dependence of the radius of gyration on protein concentration. Radii of gyration derived from Guinier plots are plotted as a function of arginine kinase concentration; the buffer is the same as in fig.1: (○) no substrate; (▲) 10 mM L-arginine; (□) 10 mM L-arginine, 10 mM ADP and 20 mM $MgCl_2$; (●) 10 mM ADP and 20 mM $MgCl_2$; (◆) 10 mM ATP and 20 mM $MgCl_2$. Least-squares lines are drawn through the data points for extrapolation to zero protein concentration.

are 21.15 ± 0.15 Å for unliganded enzyme and 19.95 ± 0.20 Å for a ternary complex enzyme–ADP–Arg. Thus, forming a complex with both substrates reduces the radius of gyration of arginine kinase by 1.20 ± 0.25 Å.

Binding nucleotides, either ATP–Mg or ADP–Mg, to form a binary complex, reduces the radius of gyration of the protein by a similar value (fig.2). On the other hand, no significant modification of the radius of gyration is observed upon binding L-arginine alone.

The normalized intensity extrapolated to zero-angle $i_n(0)$, is related to the $M_r$ of the scattering unit [11]. In our experiments, it does not depend on protein concentration. Thus, arginine kinase shows no sign of aggregation either as a function of protein concentration or upon binding ligand. The $M_r$ derived from the value of $i_n(0)$ is 39500, not significantly different from the value derived from biochemical data [7].

## 4. DISCUSSION

Our X-ray scattering measurements indicate that arginine kinase undergoes a conformational change when it binds ligands, large enough to af-

fect its radius of gyration. Similar results have been obtained on other proteins in solution by the same technique: the radius of gyration of yeast hexokinase decreases by 0.95 ± 0.24 Å when it binds glucose [12], that of yeast phosphoglycerate kinase decreases by 1.09 ± 0.34 Å when it binds ATP–Mg and 3-phosphoglycerate [13], and that of *Escherichia coli* arabinose binding protein by 0.94 ± 0.33 Å when the sugar is bound [14]. These changes are of the same importance as the one observed here (1.20 ± 0.25 Å). In the case of yeast hexokinase, the nature of the conformation change is known from the high-resolution X-ray structures of the protein crystallized either with or without glucose [3,4]. A comparison of these two structures reveals that a lobe or domain of the molecule rotates by some 10° relative to the other domain, resulting in movements of the polypeptide chain of up to 9 Å, and in closing the cleft between domains where glucose is bound. The amplitude of the change of the radius of gyration calculated from the two crystal structures is the same as observed by low-angle X-ray scattering, thus confirming the existence of domain movements in solution [12].

Though structural data are less complete in the case of phosphoglycerate kinase and of arabinose binding protein, the same mechanism is assumed to lead to the changes of the radius of gyration observed in solution [6,14], with similar functional implications. It is safe to interpret our data on arginine kinase as indicating domain motion upon ligand binding, to be eventually confirmed by its crystal structure. This puts the enzyme in line with other phosphokinases [6], from which it differs however in that nucleotide binding rather than substrate binding appears to induce the conformational change, as we find that ATP–Mg or ADP–Mg affect the radius of gyration, but not L-arginine.

Ligand-induced conformational changes have been observed in arginine kinase by UV absorption and fluorescence spectroscopy [15], and also by nuclear magnetic resonance [16]. Our results are in agreement with these studies and imply that, while effects linked to arginine binding may be local, nucleotide binding induces large scale movements such as domain motion of the sort that has been described in yeast hexokinase.

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