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MULTIPLE EFFECTS OF ANIONS ON ATP:L-ARGININE PHOSPHOTRANSFERASES

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SUMMARY

1. Anions inactivate Sipunculus and Solen arginine kinases (ATP:L-arginine phosphotransferase, EC 2.7.3.3) of molecular weight 84 000. The order of effectiveness approximates the lyotropic series of Hofmeister.

2. The anion Cl^- is a non-competitive inhibitor with respect to L-arginine and Mg^{2+}–ATP. The non-competitive inhibition is of the slope-parabolic and intercept-parabolic type.

3. The action of anions on Sipunculus arginine kinase is characterized by two steps. The first one is a fast inhibition occurring immediately after the addition of salt to enzymes and taking place even at low temperature (5°). It is common to both studied arginine kinases and to a Homarus muscle enzyme of molecular weight 43 000. The second stage is a slow decrease of the catalytic activity the extent and the rate of which depend on substrate concentration and on temperature. Concomitant with the progressive inactivation of the enzyme is a modification of the absorption spectrum of the protein: the negative peak at 288 nm observed on the difference spectrum between native and salt-treated enzymes is roughly proportional to the extent of the slow inactivation. The second step is characteristic of Sipunculus phosphokinase and is not observed with Solen enzyme nor with Homarus kinase.

4. The effect of anions on arginine kinases is thoroughly reversible by dilution or by dialysis.

5. The reactivity of cysteine groups of Sipunculus and Solen enzymes is slightly but significantly enhanced by Cl^-.

INTRODUCTION

The arginine kinases (ATP:L-arginine phosphotransferases, EC 2.7.3.3) purified from Sipunculus and Solen muscles have a molecular weight of 84 000 and consist of two subunits and of two binding sites for the nucleotide substrate ADP (ref. 1,2).

Abbreviations: DFB, 2,4-dinitrofluorobenzene; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).

Nevertheless they appear to be dissimilar in many aspects and especially in the velocities of the enzymatic reactions which differ by a 2-fold factor from one to the other.

While the comparative study of the two arginine kinases is in progress, the present paper reports the results obtained by the kinetic studies of the effect of low concentrations of anions on these enzymes. The first stage of fast inhibition is common to both kinases, the second and slower step of inactivation is particular to Sipunculus enzyme and is not observed with Solen phosphokinase. Furthermore, spectral analysis gives evidence that only the first enzyme discloses a progressive conformational change during the second stage of inactivation.

MATERIALS

ADP dilithium salt and crystalline rabbit muscle lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27) were products of British Drug Houses. ATP disodium salt and NADH disodium salt were purchased from Calbiochem. Crystalline rabbit muscle pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) type II was obtained from Sigma. 2,4-Dinitrofluorobenzene (DFB) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were products of Pierce Chemical Company. Diethylpyrocarbonate was provided by Carlo Erba. Potassium salts were products R.P. analytical grade from Prolabo, used without further purification.

Phosphoenolpyruvate was prepared by the method of SCHMIDT[3]. The silver–barium salt was converted to the potassium salt. At this point, the suspension of the product in distilled water was stirred at 0° with Dowex 50 (H^+ form) and filtrated with suction through a fritted glass funnel. The acid solution was rapidly adjusted by KOH to pH 6 and the phosphoenolpyruvate content assayed. The solution was stored at −20°.

Sipunculus, Solen (ensisensis) and Homarus arginine kinases were prepared as described[1,2,4].

METHODS

Protein concentration and molecular weight

Protein concentration was calculated from the absorbance at 280 nm using $E^{1\%}_{1cm} = 7.35$, 10, 12 for Homarus[5], Sipunculus[2] and Solen[2] arginine kinases, respectively. Molecular weight of 84 000 for Sipunculus[2] and Solen[2] arginine kinases was used.

Preparation of salt solutions

Stock neutral potassium salts solutions were prepared by dissolving the salts in distilled water. Thiocyanate solution was adjusted with KOH and acetate solution with acetic acid to pH 7.6. Perchlorate and trichloroacetate solutions were prepared by neutralization with KOH of the corresponding acids.

Sedimentation experiment

The sedimentation was made comparatively on the Sipunculus arginine kinase at 7 mg/ml in the buffer 0.01 M Tris–acetate, 10^{-4} M EDTA, 0.1 M 2-mercaptoethanol

(pH 7.4) in the presence of 0.1 M and 0.4 M KCl. A Spinco Model E ultracentrifuge equipped with an RTIC temperature control system and a Schlieren optical system was used. The sedimentation test was operated in a 12-mm double-sector cell with an An-H rotor at 60 000 rev./min at 7°.

Optical rotatory dispersion experiments

The spectropolarimeter "Spectropol I" Fica (France) was used in the wavelength range from 300 to 550 nm with a 1-cm light path quartz cell. Sipunculus and Solen arginine kinases were in the buffer 0.01 M Tris–acetate, 10^{-4} M EDTA, 0.1 M 2-mercaptoethanol (pH 7.6). Comparative ORD studies were conducted on Sipunculus arginine kinase at 2.2 mg/ml 20° and on Solen enzyme at 2.53 mg/ml 15°, in the absence and in the presence of salt, 0.3 and 0.4 M KCl, respectively.

Enzyme assay

Arginine kinase activity was measured in the forward reaction estimating ADP by the coupled enzyme method (pyruvate kinase and lactate dehydrogenase)[6] with a Cary Model 14 thermostated recording spectrophotometer set at 340 nm.

A stock solution, containing Tris–acetate (pH 7.6), Mg^{2+}–ATP, KCl, magnesium acetate, NADH, phosphoenolpyruvate, lactate dehydrogenase, pyruvate kinase, was preincubated 20 min at 25° and then in the ice bath, so as to stabilize the solution by converting into ATP the trace amount of ADP usually contained in commercial ATP samples. Enzyme solution was diluted in the buffer 0.01 M Tris–acetate, 0.1 M 2-mercaptoethanol (pH 7.6).

Assay was carried out in a 1-ml cell of 1-cm light path with the following mixture: 100 mM Tris–acetate (pH 7.6), 10 mM KCl, 1 mM magnesium acetate, 0.15–0.20 mM NADH, 1 mM phosphoenolpyruvate, 30 μg lactate dehydrogenase, 30 μg pyruvate kinase, substrates (Mg^{2+}–ATP, L-arginine hydrochloride), potassium salts and reagents (DTNB, DFB), as indicated in the text. Blank cell contained distilled water.

The sample cell and the enzyme solution were equilibrated 10 min at the desired temperature. The reaction was started by addition of 100 μl of enzyme solution to 0.9 ml of the medium. After rapidly mixing the solution, the decrease in absorbance was recorded. The initial rate of oxidation of NADH corresponding to the formation of ADP was linear.

Calculations were made by the use of an extinction coefficient at 340 nm of $6.22 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ for NADH. An unit of enzyme activity was defined as the amount of enzyme which releases 1 μmole of ADP per min under the described conditions, while the specific activity was expressed as units per mg of protein.

Slow inhibition stage studies

These studies were performed by assaying enzyme activity as just described, but with the modification that blank cuvet contained all components except the arginine kinase. As the enzyme activity declined slowly in the presense of salt (see RESULTS), to ascertain that this was not an effect of thermic denaturation, a control was made with the enzyme without salt. The activity of the control was constant during the same time.

Ultraviolet difference spectra measurements

Ultraviolet difference spectra were measured in matched pair quartz cells of 1 ml capacity and 0.4375 cm light path in the wavelength range 300–240 nm with a Cary Model 15 thermostated recording spectrophotometer.

Titration of cysteinyl groups

A blank cuvet contained all the components except enzyme. After addition of arginine kinase solution to the medium and rapid mixing, absorbance was immediately recorded with a Cary Model 14 spectrophotometer set at 412 nm for DTNB and 335 nm for DFB experiments. Calculations were made by the use of the extinction coefficients of $13.6 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ for *p*-nitrothiophenol anion[7] and of $9.6 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ for *S*-dinitrophenylcysteine[8].

Titration of histidyl groups

The method of ELÖDI[9] was used for the titration of histidyl groups of arginine kinases at 15°, $1 \cdot 10^{-5}$ M enzyme, 0.01 M Tris–acetate (pH 7.0), $2 \cdot 10^{-4}$ M diethylpyrocarbonate, in the absence of salt or in the presence of 0.1 M KCl for Sipunculus and 0.3 M KCl for Solen enzymes.

RESULTS

Inhibition by anions

The salts used were potassium salts in order to eliminate the interaction of the effect of cations. The tested anions decrease the activity, the Sipunculus enzyme

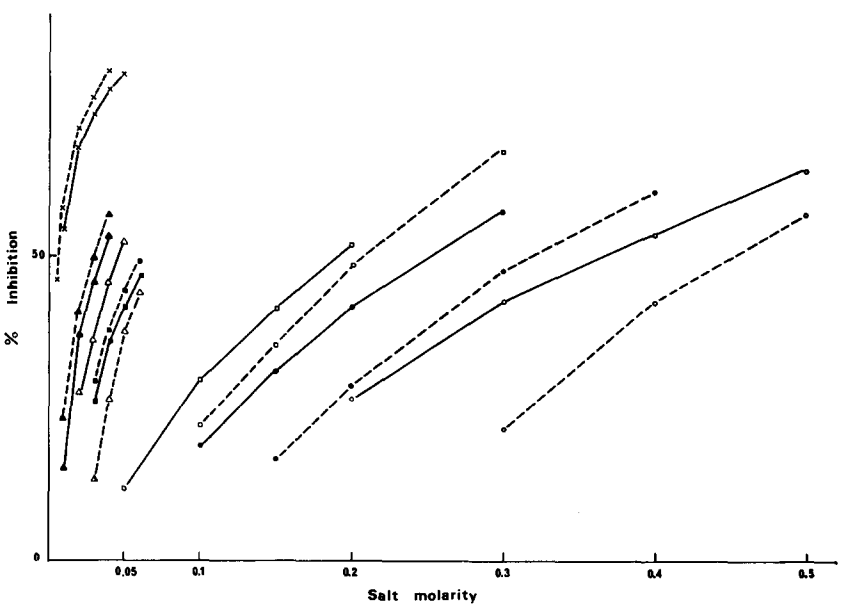


Fig. 1. Inhibition of Sipunculus and Solen arginine kinases by monovalent anions. Temp. 10°, 1 mM arginine, 0.3 mM Mg^{2+}–ATP; ——, Sipunculus arginine kinase; — — —, Solen arginine kinase; ○, acetate; ●, chloride; □, bromide; ■, thiocyanate; △, trichloroacetate; ▲, perchlorate; ×, nitrate.

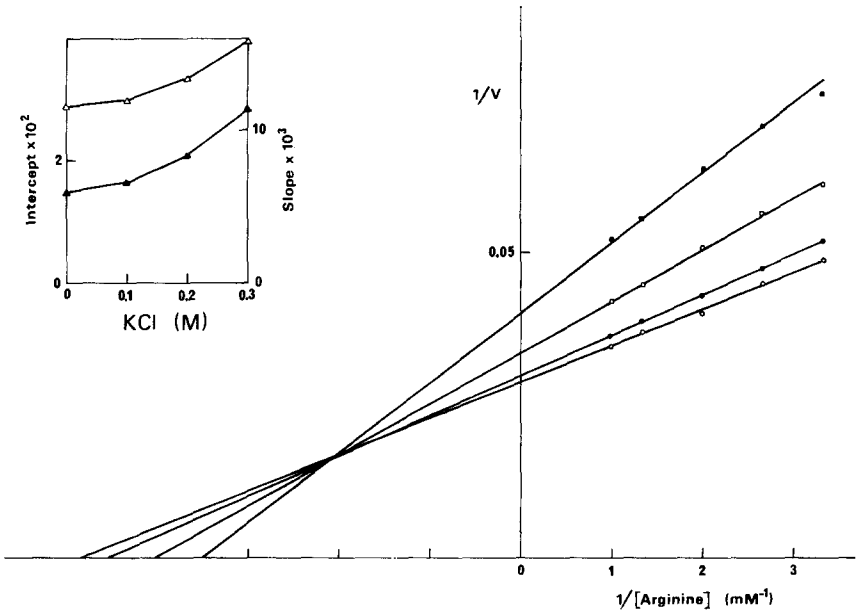


Fig. 2. Kinetics of the forward reaction of Sipunculus arginine kinase. Temp. 10°, 5 mM Mg^{2+}-ATP as fixed substrate and 0.3, 0.375, 0.5, 0.75, 1 mM arginine as variable substrate. Primary plots: ○—○, 0 KCl; ●—●, 0.1 M KCl; □—□, 0.2 M KCl; ■—■, 0.3 M KCl. Secondary plots: △—△, ordinate intercepts; ▲—▲, slopes.

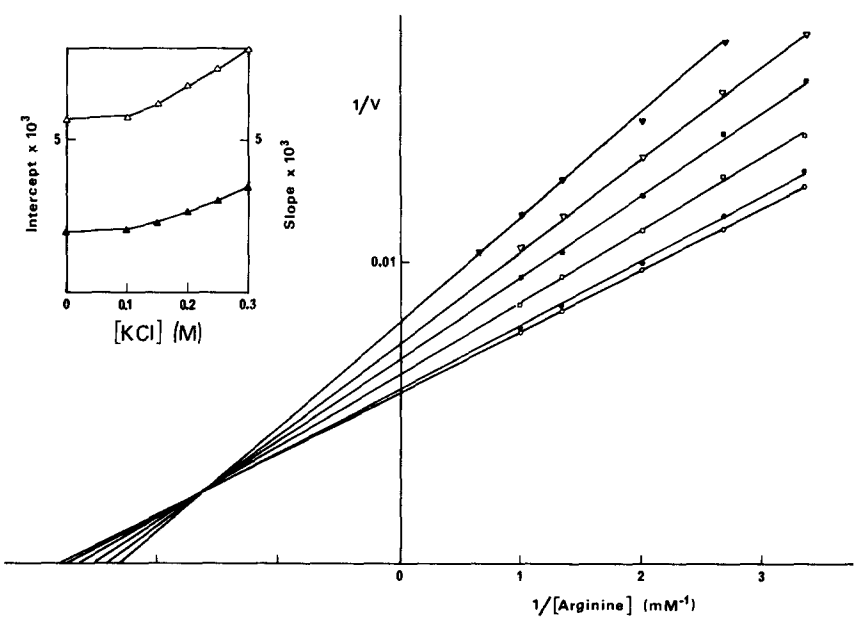


Fig. 3. Kinetics of the forward reaction of Solen arginine kinase. Temp. 10°, 5 mM Mg^{2+}-ATP as fixed substrate and 0.3; 0.375, 0.5, 0.75, 1, 1.5 mM arginine as variable substrate. Primary plots: ○—○, 0 KCl; ●—●, 0.1 M KCl; □—□, 0.15 M KCl; ■—■, 0.2 M KCl; △—△, 0.25 M KCl; ▲—▲, 0.3 M KCl. Secondary plots: △—△, ordinate intercepts; ▲—▲, slopes.

being slightly more susceptible to the action of anions than the Solen enzyme (Fig. 1). The order of effectiveness of inhibition by monovalent anions is nearly that of the lyotropic series of Hofmeister:

$$CH_3COO^- < Cl^- < Br^- < SCN^- \simeq CCl_3COO^- < ClO_4^- < NO_3^-$$

The anion effect is reversible by dialysis and by dilution. Thus, Sipunculus arginine kinase treated with 0.1, 0.2 M KCl, 30 min at 22°, then diluted 200-fold, or treated with 0.09, 0.18, 0.27 M KCl, 10 min at 30°, then dialysed at 5° overnight, regained its full activity.

Sedimentation and ORD studies

In the presence of 0.4 M KCl, the concentration at which Sipunculus arginine kinase activity is half reduced, the sedimentation coefficient is the same as that of the enzyme at 0.1 M KCl. At the latter KCl concentration, the enzyme is not dissociated[2]. Thus the possibility of a dissociation of the enzyme is precluded.

The features of ORD peculiar to each of these enzymes undergo no modification after treatment of Sipunculus enzyme with 0.3 M KCl and Solen enzyme with 0.4 M KCl.

Kinetics of inhibition by anions

(1) *Immediate inhibition stage.* For both enzymes, the inhibition is of non-competitive type with respect to arginine (Figs. 2 and 3) and ATP. An increase of the Michaelis constants for both substrates was observed; furthermore, a decrease of the maximal velocity was seen (Table I). Secondary plots of slopes (K_m/v_{max}) and intercepts ($1/v_{max}$) *versus* KCl concentration are parabolic. Thus, the inhibition is of a parabolic non-competitive type, according to CLELAND[10]. In such a system, a single inhibition constant does not exist for each anion.

The pK_m curves with respect to guanidine substrate of native enzyme and 0.2 M KCl treated enzyme disclose no difference in their enzyme–arginine complex and free enzyme pK values.

TABLE I

EFFECT OF Cl^- ON KINETIC PARAMETERS OF SIPUNCULUS AND SOLEN ARGININE KINASES

Sipunculus arginine kinase: Enzyme 6.6 μg/ml in (0.01 M Tris–acetate, 0.1 M 2-mercaptoethanol) pH 7.6 buffer, 0, 0.1, 0.2 M KCl. Enzyme assays at 25°; 3 mM arginine as fixed substrate and 0.3, 0.375, 0.5, 0.75, 1, 1.5 mM Mg^{2+}–ATP as variable substrate. In these experiments the kinetic parameters are determined on the inhibited enzyme after 30-min incubation with KCl at 25°. Solen arginine kinase: Enzyme 2.5 μg/ml in (0.01 M Tris–acetate, 0.1 M 2-mercaptoethanol) pH 7.6 buffer; 0, 0.2, 0.3 M KCl, at 0°. Enzyme assays at 30°; 10 mM arginine as fixed substrate and 0.3, 0.375, 0.5, 0.75, 1, 1.5 mM Mg^{2+}–ATP as variable substrate.

KCl (M)	*Sipunculus arginine kinase*		*Solen arginine kinase*	
	Mg^{2+}–ATP Apparent K_m (mM)	*v_{max} (μmoles/mg per min)*	*Mg^{2+}–ATP Apparent K_m (mM)*	*v_{max} (μmoles/mg per min)*
0	0.5	135	0.8	500
0.1	0.9	105	–	–
0.2	1.1	65	1.3	330
0.3	–	–	1.8	260

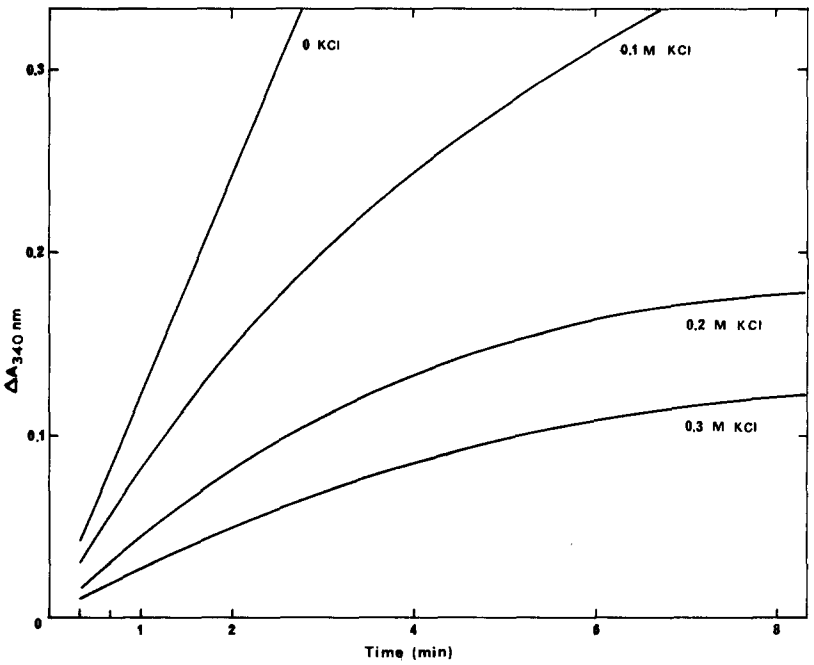


Fig. 4. Cl^- concentration dependence of the slow inhibition stage of Sipunculus arginine kinase. Absorbance at 340 nm monitored with time. Enzyme 1 μg/ml, temp. 30°, 0.3 mM Mg^{2+}–ATP, 0.3 mM arginine, 0, 0.1, 0.2, 0.3 M KCl, 1 inch/min chart speed.

(2) *Slow inhibition stage.* With Sipunculus enzyme, the fast and partial inhibition promoted by anion is followed by a very slow decline of enzymic activity. This second step the extent and the rate of which depend on substrates concentration has been studied as a function of salt concentration and of temperature between 5 and 25°. At 30° the rate of inhibition increases considerably with the enhancement of KCl concentration (Fig. 4). In the presence of non-saturating substrates, the slow decrease of activity, not yet disclosed after 45 min at 5° and 10°, becomes more marked from 15 to 25° (Fig. 5). At 25° it is noticeable in 2 min. In contrast, at the saturating substrates concentration and with 0.4 M KCl which immediately reduces half the enzymatic activity, the slow stage of inactivation is not perceivable after 2 min at 25°. The same data were obtained also with SCN^- and CCl_3COO^-.

In the same conditions the slow stage of inhibition was not observed with Solen enzyme nor with arginine kinase of molecular weight 43 000 from Homarus muscle.

Because of this phenomenon observed with Sipunculus enzyme, kinetic studies of the immediate inhibition stage produced by salts should be performed at 10°, the temperature at which the slow stage of inactivation was not perceivable.

Spectrophotometric analysis of the interaction of enzymes with anions

The binding of L-arginine and of Mg^{2+}-ADP to Sipunculus and Solen arginine kinases gives rise to characteristic difference spectra[2] quite similar to those observed with Homarus muscle enzyme[11]. In the case of the two first enzymes, the main features of these difference spectra are not modified by the action of anions. The

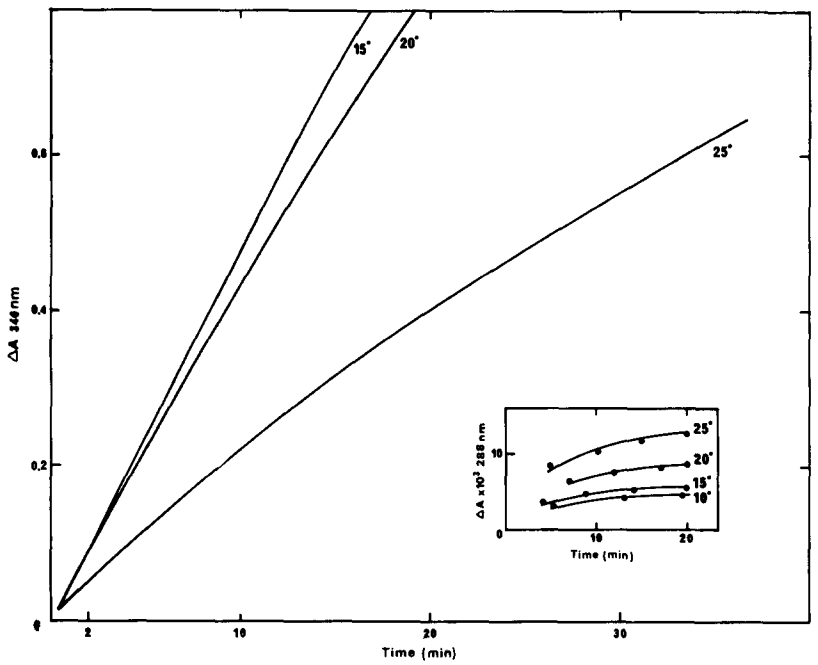


Fig. 5. Temperature dependence of chloride effect on the slow inhibition stage of Sipunculus arginine kinase. Absorbance at 340 nm monitored with time. Enzyme 1 μg/ml, 0.3 M KCl, 0.3 mM Mg^{2+}–ATP, 1 mM arginine, 15°, 20°, 25°, $\frac{1}{4}$ inch/min chart speed. The inset shows the temperature dependence of chloride effect on difference spectra between native Sipunculus arginine kinase and salt treated enzyme. Enzyme 1.8 mg/ml, 0.01 M Tris–acetate (pH 7.6), 0.326 M KCl. In the vertical axis, the absorbance difference ΔA was measured at 288 nm.

amplitude of the enzyme–arginine and enzyme–ADP–Mg^{2+} difference spectra, however, decreases with the rise of Cl^- concentration (Fig. 6).

Furthermore, as is shown in Fig. 7, the difference spectrum between native Sipunculus arginine kinase and the salt treated protein is characterized by a negative peak at 288 nm and an attending shoulder at 280 nm. The modification of the absorption spectrum of the protein was not observed with Solen enzyme nor with Homarus arginine kinase. This spectrum could be interpreted as a perturbation at the region of absorption of tyrosine and of tryptophan residue. It is not specific for a given ion and is identical for the examined ions Cl^-, SCN^-, CCl_3COO^-. With Cl^-, its magnitude increases with time (Fig. 8) and reaches a maximum in 20 min. The value of the maximum increases with the rise of temperature between 10 and 25° (Fig. 5, inset) and depends on salt concentration. (Fig. 9) This spectral modification was not seen with 0.326 M KCl when the enzyme has been previously in contact with $0.92 \cdot 10^{-2}$ M arginine.

The effect of anions on the absorption spectrum of the enzyme seems no doubt to correspond, with regard to time, to the second stage of slow inhibition. In fact, the perturbation of the spectrum does not appear in the conditions for which the slow stage of inactivation is not seen. It is the case for the Solen and Homarus enzymes which do not display the second stage of inhibition. It is also the case for Sipunculus enzyme when protected by saturating substrates. Moreover, this interpretation is

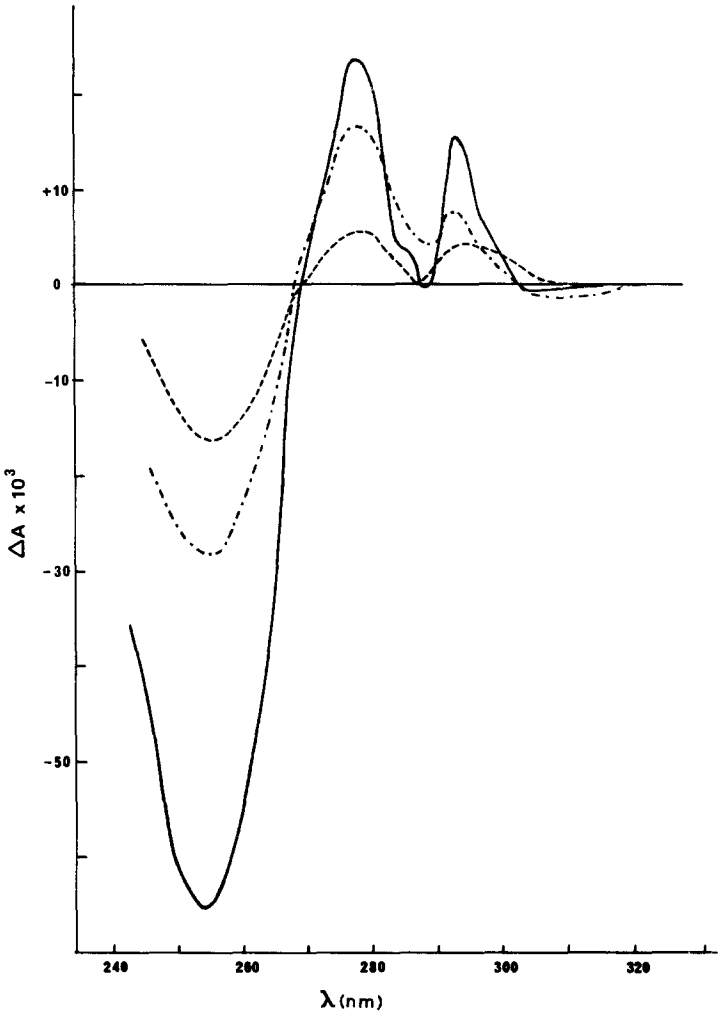


Fig. 6. Difference spectra of Mg^{2+}–ADP–Solen arginine kinase complex in the absence and the presence of KCl. Enzyme 1.54 mg/ml, 0.01 M Tris–acetate, $1 \cdot 10^{-4}$ M EDTA (pH 7.6), 0.138 mM Mg^{2+}–ADP, temp. 15°; ——, 0 KCl; —·—, 0.2 M KCl; — — —, 0.4 M KCl.

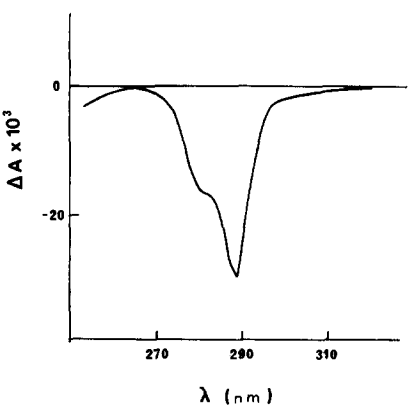
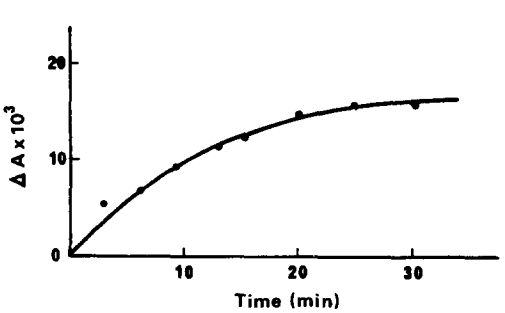


Fig. 7. Difference spectrum between native Sipunculus arginine kinase and Cl^--treated enzyme. Enzyme 1.31 mg/ml, 0.01 M Tris–acetate, 0.4 M KCl (pH 7.6), temp. 22°.



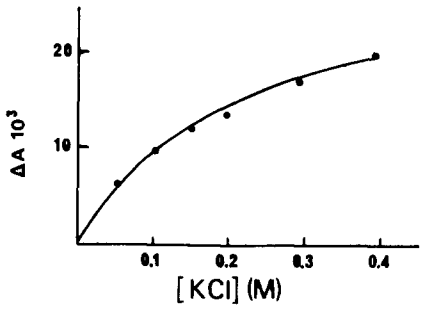


Fig. 8. Time dependence of chloride effect on difference spectra between Sipunculus arginine kinase and salt-treated enzyme. Enzyme 1.80 mg/ml, 0.01 M Tris–acetate (pH 7.6), 0.367 M KCl, temp. 20°. In the vertical axis, the absorbance difference ΔA was measured at 288 nm.

Fig. 9. Cl^- concentration dependence of difference spectra between Sipunculus arginine kinase and salt treated enzyme. Enzyme 1.95 mg/ml, 0.01 M Tris–acetate (pH 7.6). Temp. 20°. In the vertical axis, the absorbance difference ΔA was measured at 288 nm.

strengthened by the fact that the intensity of the spectral effect and the rate of the slow inhibition stage are parallel functions with respect to temperature.

Reactivity of cysteine and histidine groups

The fast inhibition of arginine kinases by anions suggests that the conformation at the active site should be more or less modified and the reactivity of essential amino acid residues should be changed. As essential cysteine and histidine residues have been found in rabbit muscle creatine kinase and Homarus muscle arginine kinase[12,13], the eventual effect of anions on the reactivity of cysteine and histidine groups was examined.

The Sipunculus and Solen arginine kinases possess, respectively, 12 and 8 total cysteine groups, all of which are reactive toward DTNB (ref. 1,2). With Sipunculus enzyme, DTNB at 13.5 equivalents titrates in 15 sec 4.2 –SH and 5.5 –SH in the absence and in the presence of 0.4 M Cl^-, and DFB at 11.8 equivalents titrates in 6 min 1.9 –SH and 3.2 –SH in the absence and in the presence of 0.4 M Cl^-. With Solen enzyme, DTNB at 6.6 equivalents titrates in 15 sec 3.0 –SH of the native enzyme and 3.4 –SH in the presence of 0.4 M Cl^-, DFB at 11.8 equivalents titrates in 6 min 0.6 –SH of the native enzyme and 0.9 –SH in the presence of 0.4 M Cl^-.

The enhancement of cysteine groups reactivity (Fig. 10) of both enzymes with 0.4 M KCl is not in fact important, but it appeared significant because of the reproducibility of results obtained in several experiments.

The kinetics of carbethoxylation of histidine groups performed in parallel with enzyme assays showed a tiny variation in the effect of Cl^- at 0.1 M and 0.3 M on Sipunculus and Solen enzymes, respectively.

The reactivity of cysteine and histidine groups, as a parameter indicating conformational change, appeared of little significance in this work, because of the great reactivity of these groups in both enzymes in the native state.

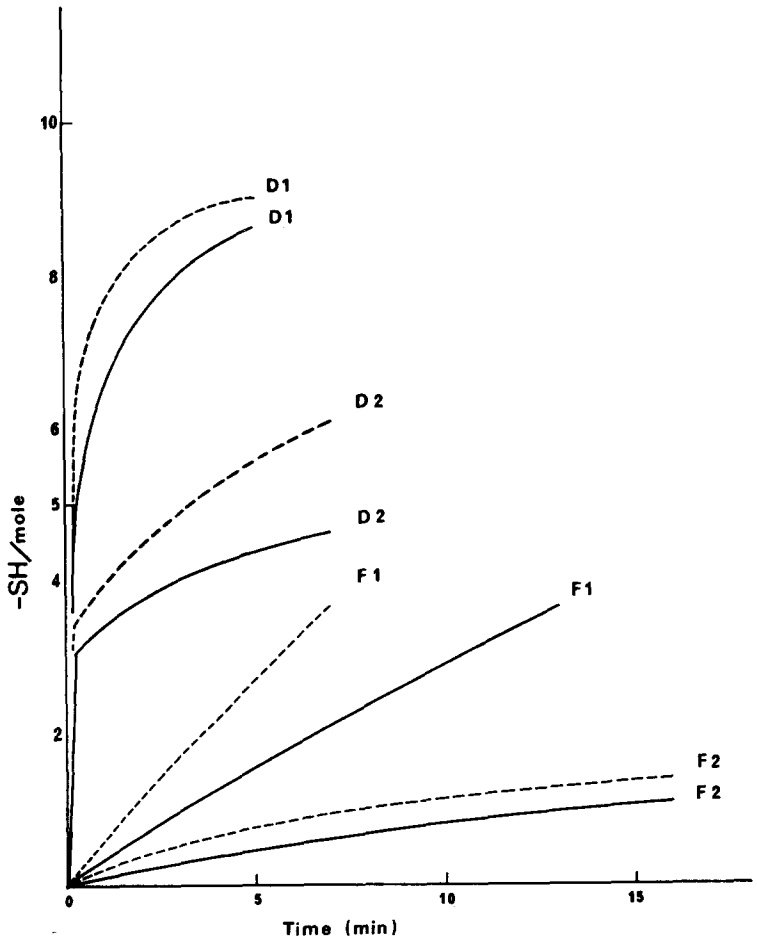


Fig. 10. Enhancement of cysteine groups reactivity of Sipunculus and Solen arginine kinases by chloride. Temp. 10°. ——, o KCl; — — —, 0.4 M KCl; D1: $6.05 \cdot 10^{-6}$ M Sipunculus enzyme, $8.16 \cdot 10^{-5}$ M DTNB; D2: $1.2 \cdot 10^{-5}$ M Solen enzyme, $8 \cdot 10^{-5}$ M DTNB; F1: $1.2 \cdot 10^{-5}$ M Sipunculus enzyme, $1.42 \cdot 10^{-4}$ M DFB; F2: $1.2 \cdot 10^{-5}$ M Solen enzyme, $1.42 \cdot 10^{-4}$ M DFB.

DISCUSSION

The mechanism by which Cl^- affects the catalysis reaction of the two arginine kinases is of a complex type, since a non-competitive inhibition with parabolic slopes and intercepts is involved. The decrease of the amplitude of the enzyme–arginine and enzyme–ADP–Mg^{2+} difference spectra (Fig. 6) corroborates the results obtained in kinetic studies although no variation in the main features of substrate binding spectra is observed.

From previous works, it arises that this mode of action of ions is not specific for a type of protein structure but is characteristic of the ions effect. It is worth quoting in particular the kinetic studies of HEYDE AND MORRISON[14] on creatine kinase and those of DENBURG AND MCELROY[15] on firefly luciferase.

These authors observe also a non-competitive inhibition in which the complexity of the type of inhibition is more or less important. Thus, the affinity of creatine kinase with respect to four substrates is affected, whereas the affinity of luciferase decreases only for Mg^{2+}–ATP, the K_m for luciferine being unchanging. Also, intercepts and slopes could be parabolic or linear or of mixed type. The degree of complexity seems to be connected with the structure of protein and with salt and substrates concentration. The substrates would act as protectors against the inhibition by ions stabilizing the protein structure.

The order of effectiveness of ions on the catalytic activity of both arginine kinases does not exhibit a striking difference. But the Sipunculus enzyme shows a susceptibility toward ions higher than that of Solen enzyme. The perturbation of the enzyme spectrum and the second stage of inactivation, concomitant processes following the stage of fast inhibition and evolving slowly, are characteristic of Sipunculus enzyme. This set of facts expresses the structural divergences existing between the two enzymes. This is in agreement with the views of WARREN *et al.*[16]. According to these authors the effect of salts on the enzymatic activity may be used as a very sensitive parameter to estimate a conformational change. More precisely, we think that the effect of anions in its early manifestation on the kinetic constants, reflects some structural modification of the active center. A difference in the sensitivity toward ions may be a sign fo structural dissimilarities, possibly very small, in the area of the active center of these two enzymes. Of course an eventual direct interaction of ions with the active site does not implicate as such an exclusive localization of this action.

But if conformational changes should take place in other regions of proteins they should be limited and are not disclosed by ORD nor by sedimentation investigations. Furthermore the temperature dependent perturbation of the Sipunculus protein spectrum is a late event associated with the stage of slow inactivation and not to that of fast inhibition.

The modification of the absorption spectrum of Sipunculus enzyme, perceivable with KCl concentrations as low as 0.05 M, is an originality of this protein structure. We have not found any similar case in the literature. We could only quote a study on the variations, otherwise very important, of the spectrum of ribonuclease by the action of LiBr (ref. 17,18). Yet this effect has been studied only at salt concentrations higher than 3 M.

A set of facts suggest that the primary effect of anions is directed at the active site of enzymes. The immediate inhibition takes place even at 5° and varies markedly with substrate concentration. The very efficient protection of enzyme by substrates against the inactivation may be stressed. Finally the small but reproducible enhancement of the reactivity of cysteine groups in the presence of Cl^- is significant although these groups are highly reactive in both native arginine kinases.

The ignorance of the nature of the perturbation induced by ions limits the exploitation of the results provided by these studies. The whole set of papers[15,19–21] studying the susceptibility toward ions of enzymic proteins suggests that the inhibition mechanisms can not be explained purely in terms of electrostatic interaction[19]. The hypothesis which seems to account for the observed facts best is based on the importance of hydrophobic bonds in the maintenance of the active conformation of proteins. The action of ions would occur specifically at the level of the water struc-

ture[22,23]. The dis-structuration of water surrounding non-polar groups leads to a breaking of hydrophobic bonds[24,25]. This conception would justify to a great extent the sensitivity toward ions of the catalytic function of Sipunculus and Solen enzymes. In fact, there is a great similarity between the two substrates binding sub-sites of these two enzymes[2] and the same ones of Homarus arginine kinase[11,26,27]. In the last case, the binding to the enzyme of L-arginine, L-isoleucine, L-valine, but not that of L-leucine, L-alanine discloses a characteristic difference spectrum suggesting the presence of a particular hydrophobic sub-site at the active center[11,26,27]. This set of data would involve an anion action directed to a similar hydrophobic sub-site of the active center of Sipunculus and Solen enzymes.

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