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STUDIES ON THE PARTIAL EXCHANGE AND OVERALL REACTIONS CATALYZED BY NATIVE AND MODIFIED ARGININE KINASE FROM *HOMARUS VULGARIS* MUSCLE

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SUMMARY

Initial velocity and partial exchange studies are performed on arginine kinase (ATP:L-arginine phosphotransferase, EC 2.7.3.3) from *Homarus vulgaris* muscle. The steady-state kinetic patterns suggest a reaction mechanism proceeding via interconversion of ternary complexes, but we can also observe partial exchange reactions between ATP and ADP or arginine phosphate and arginine. Properties of these exchange reactions are investigated. In addition, arginine kinase is the only phosphagen kinase studied which catalyses this partial exchange.

Comparison between partial exchange rates related to native and specifically inhibited arginine kinase leads to confirmation of our results previously obtained concerning the role of several essential amino acid residues in the active site: dansylation of one lysyl residue or carboxymethylation of one cysteinyl residue prevents the formation of guanidine–enzyme complexes, thus only the arginine phosphate–arginine exchange is abolished. Carbethoxylation of the histidyl residue implicated in the catalytic process suppresses the two partial exchange reactions.

INTRODUCTION

In earlier communications[1,2] different authors have concluded from initial velocity patterns that arginine kinase (ATP:L-arginine phosphotransferase, EC 2.7.3.3) catalyses a sequential mechanism reaction, occurring by the interconversion of ternary complexes, and that the true nucleotide substrate is the ATP–Mg^{2+} complex[3–5]. In the same way, MORRISON[2] had observed, with arginine kinase from *Jasus verreauxi*, a slow but real partial isotopic exchange reaction between arginine phosphate and arginine or ATP and ADP, in the absence of other substrates. The interpretation of this author, based on these findings, suggested the existence of an intermediary mechanism involving a two-reaction flux.

In this paper, initial velocity studies have been reinvestigated on arginine kinase

Abbreviation: DNS, *N,N*-dimethyl-1-aminonaphthalene-5-sulphonate.

from another source, *Homarus vulgaris*, and partial isotopic exchange reactions of this enzyme were studied.

In the first part of this paper, we detail the importance and characteristics of these exchange reactions, and in the second part we extend these studies to chemically modified arginine kinase. In a previous communication new data, obtained by means of difference spectrophotometry on specifically modified enzymes, were reported on the role of several essential amino acid residues (cysteine, lysine, histidine)[6]. The presence of these residues at the active site was demonstrated previously by labelling with specific reagents[7–10].

MATERIAL AND METHODS

Enzymes

Arginine kinase from *H. vulgaris* is prepared according to the method of DER TERROSSIAN *et al.*[11], taurocyamine kinase (ATP:taurocyamine phosphotransferase, EC 2.7.3.4) from *Arenicola marina* as described[12], and creatine kinase (ATP:creatine phosphotransferase, EC 2.7.3.2) from rabbit muscle according to the method of KUBY[13] (Procedure B). Their specific activities are 230, 170 and 80, respectively.

Substrates

L-Arginine (99% purity) is provided by Hoffmann La Roche, uniformly ^{14}C-labelled L-arginine (specific activity 49 mC/mmole) by Commissariat à l'Energie Atomique, Saclay; arginine phosphate is prepared as previously described[14].

The nucleotides used are obtained from Calbiochem and their aqueous solutions adjusted to pH 7.0 by addition of NaOH (0.1 M). Their concentrations are determined spectrophotometrically from their molar absorbance[15].

[^{14}C]ADP is purchased from Schwartz (specific activity 32.8 mC/mmole).

Inhibitors

Dansyl chloride is a Pierce Chemical Co. product; it is used without further purification. Diethylpyrocarbonate is from Carlo Erba; $K_2S_4O_6$ is a Merck product and 5,5'-dithiobis-2-nitrobenzoic acid a Pierce Chemical Co. product. Dithiothreitol is obtained from Nutritional Biochemicals Corporation. All the other products used are of the best analytical grade.

Protein concentration

Protein concentration of the enzymes is obtained from their absorbance at 280 nm[16,17]. Molar concentrations are calculated by assuming molecular weights of respectively 43 000 for arginine kinase[18,19] ($E^{1\%}_{1cm} = 7.35$), 81 000 for creatine kinase[20] ($E^{1\%}_{1cm} = 8.6$) and 81 000 for taurocyamine kinase[19,21] ($E^{1\%}_{1cm} = 11.4$).

Preparation of chemically modified phosphagen kinases

Sulphydryl labelling. Labelling of the essential cysteinyl residue is performed under the experimental conditions detailed in a previous paper[22]. 1.5 mg protein is allowed to react 35 min in 0.1 M Tris–acetate buffer (pH 7) with iodoacetamide (1.5 mM) at room temperature. Purification of 100% inactivated *S*-carboxymethyl arginine kinase is achieved by exhaustive dialysis against 0.1 M Tris–acetate buffer, pH

7.2. The inactivated enzyme was found by the ELLMANN[23] method to possess only two alkylated sulphydryl groups, including the essential one. Indeed, as judged by difference spectra, binding of L-arginine does not occur on the modified enzyme, whereas interaction of nucleotide substrates still occurs[22]. Further, no conformational change is observed by the optical rotatory dispersion method (unpublished results).

DNS labelling. The ε-amino group of lysine is dansylated as reported by KASSAB *et al.*[9]. Arginine kinase (10 mg/ml), in 0.05 M $NaHCO_3$ buffer, pH 8, is allowed to react 60 min with dansyl chloride (7 mM) at 0°. The inhibited dye-labelled proteins are purified by molecular sieving on Sephadex G-25 as previously described[6]. The number of *N,N*-dimethyl-1-aminonaphthalene-5-sulphonate (DNS) groups present (1.5 moles DNS per mole enzyme) is measured by their absorption at 335 nm[24]. The modified enzyme studied is 100% inhibited and possesses all its sulphydryl groups free. Further, optical rotatory dispersion does not reveal any conformational change after the labelling of the essential lysyl residue[6]. But by difference spectroscopy we can observe the spectral changes described previously on interaction of this modified protein with its substrates, *i.e.* abolition of the guanidine substrates induced difference spectrum and perturbation of the difference spectrum associated with the binding of ADP[6].

Protein carbethoxylation. Arginine kinase (10 mg/ml) in 0.05 M phosphate buffer (pH 6.1) is allowed to react with diethyl pyrocarbonate ($3 \cdot 10^{-3}$ M) for 6 min at room temperature. The preparations are freed from excess reagent by filtration on Sephadex G-25 equilibrated with 0.01 M Tris–acetate buffer (pH 7.1). The number of carbethoxyimidazole groups is calculated from their specific difference absorption at 240 nm[25].

The samples studied are 90–95% inhibited and labelled at an average of 1.5–1.8 residues per mole protein. As mentioned before[6], no conformational changes are observed by optical rotatory dispersion measurement, whereas difference spectroscopy shows that nucleotide and guanidine substrates are always able to bind to this modified enzyme without transphosphorylation[6].

Partial isotopic exchange reactions

ATP–ADP exchange. In a typical experiment, the reaction mixture contains in 0.5 ml: 0.05 M Tris–acetate buffer (pH 8.0), 0.25 μmole ATP, 0.5 μmole magnesium acetate and 1 nmole enzyme. After equilibration for 3 min at the desired temperature, the reaction is started by the addition of [^{14}C]ADP (0.25 μC).

The exchange reaction is followed with respect to time and 50-μl aliquots of reaction mixture are taken off for the determination of the amount of [^{14}C]ATP produced. The reaction is stopped simply by application of these aliquots to DEAE-cellulose paper (Whatman DE 81 paper type). The reference contains all the components of the reaction mixture except magnesium acetate.

Arginine phosphate–arginine exchange. The reaction mixture for this second exchange reaction contains 0.5 μmole arginine phosphate and 2 nmoles enzyme in 0.5 ml 0.05 M Tris–acetate buffer (pH 8.0). The reaction is started as previously described by addition of uniformly ^{13}C-labelled arginine (0.5 μC) and stopped by spotting 50-μl aliquots on Whatman DE 81 paper.

Nucleotide diphosphokinase reaction. This reaction is performed in 0.05 M Tris–

acetate buffer (pH 8.0) at 25°. In a typical experiment, the reaction mixture contains in 0.5 ml buffer: 1 nmole enzyme, 0.5 mM ADP, 0.25 μC [^{14}C]ADP, 1 mM magnesium acetate. The reaction is started by the addition of GTP (0.5 mM) and stopped by spotting aliquots on Whatman DE 81 paper.

ATP–ADP and arginine phosphate–arginine separation

Chromatographic separation between ATP and ADP is performed according to ref. 2 using 0.6 M ammonium formate buffer (pH 3.0) as solvent. Nucleotides are detected by their fluorescence under ultraviolet light.

Separation of arginine from arginine phosphate is obtained by ascending chromatography on Whatman DE 81 paper with $5 \cdot 10^{-3}$ M NaCl as the developing solvent. In each case radioactive bands, detected by means of a radiochromatogram Scanner (Packard model 7200), are cut out and the radioactivity measured directly by liquid scintillation in a Packard Tri Carb scintillation spectrometer model 3003.

Determination of isotopic exchange rate

In order to obtain an apparent rate constant, and therefore the rate of the partial exchange reactions, without knowing the exact nature of the different steps, $\log x_e/(x_e - x)$ or $1/(x_e - x)$ is plotted *versus* time so that a linear representation of radioactivity incorporation *versus* time in the first stage of the reaction can be established. x_e represents radioactivity at equilibrium and x at time t.

Determination of initial velocity

The enzyme reactions are carried out in 0.1 M glycine–NaOH buffer (pH 8.5). Initial velocity is obtained by two methods:

In the first procedure, velocity is calculated by measuring the rate of incorporation of ^{14}C into arginine phosphate from [^{14}C]arginine. The experimental procedure is the same as for exchange reaction studies. The transphosphorylation reaction is stopped after 5 min by application of the mixture to Whatman DE 81 paper.

In the second procedure, the rate of release of ADP from ATP is determined spectrophotometrically by enzymic coupling with two enzymes [pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) and lactate dehydrogenase (L-lactate:NAD$^+$ oxidoreductase, EC 1.1.1.27)] according to the method elaborated by THIEM *et al.*[26]. Initial velocity is obtained from the slope of the tangent related to the decrease in NADH absorbance.

RESULTS

(1) Initial velocity studies on arginine kinase

By means of the two methods described above, the study of initial rates with different substrates leads to identical results. As is shown in Fig. 1 a low range of substrate concentrations is chosen; with higher substrate concentrations[2] slope changes in LINEWEAVER–BURK plots would not be detectable. Analysis was made graphically according to FLORINI[27] (Fig. 2) to evaluate Michaelis constants. According to CLELAND's[28] nomenclature, and if a represents arginine and b refers to ATP–Mg^{2+}, we obtain: $K_a = K_{ia} = 0.65$ mM and $K_b = K_{ib} = 0.55$ mM.

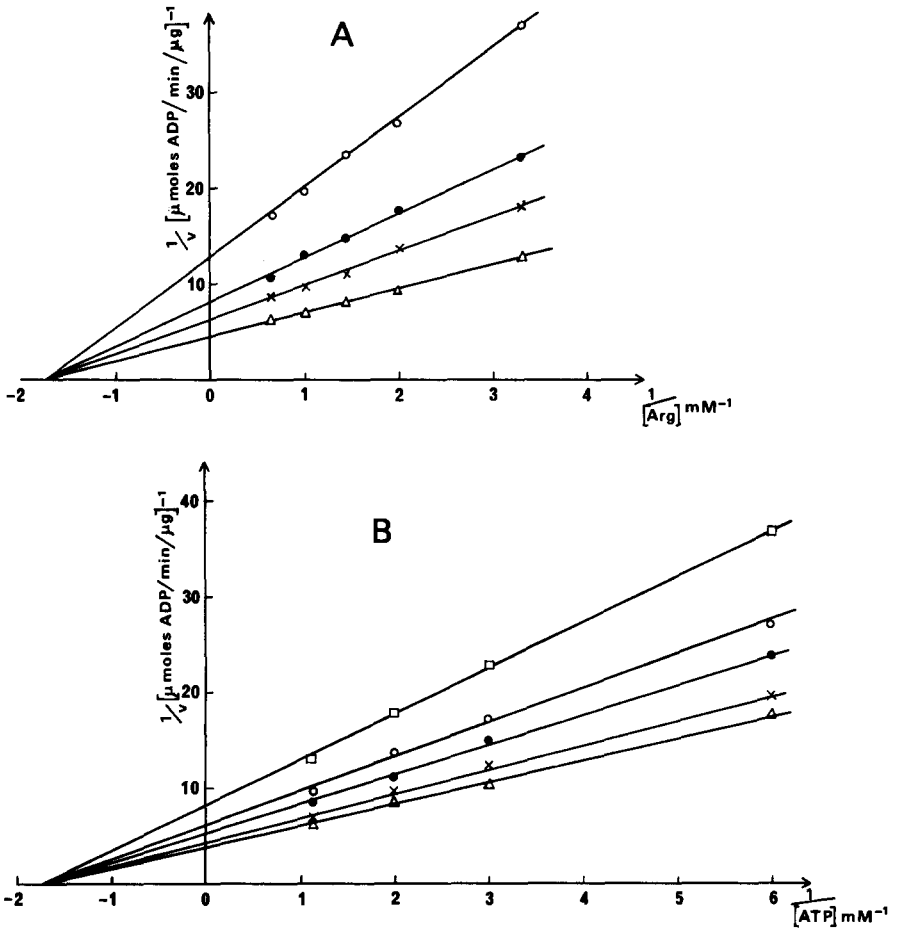


Fig. 1. Lineweaver–Burk representation of initial velocities measurements at pH 8.5 and 25°. A. $1/v_i$ *versus* 1/[arginine] at fixed concentration values of ATP–Mg^{2+}: △—△, 0.89 mM; ×—×, 0.50 mM; ●—●, 0.34 mM, ○—○, 0.17 mM. B. $1/v_i$ *versus* 1/[ATP–Mg^{2+}] at fixed concentration values of arginine: △—△, 1.50 mM; ×—×, 1.00 mM; ●—●, 0.70 mM; ○—○, 0.50 mM, □—□, 0.30 mM. In all cases free [Mg^{2+}] is fixed at 1 mM.

(II) Isotopic exchange reactions

If a partial exchange reaction occurs in the reactions catalysed by arginine kinase, we can write it as follows:

$$E + \text{ATP–Mg}^{2+} \leftrightharpoons EP + \text{ADP–Mg}^{2+}$$
$$E + \text{Arg} \sim P \leftrightharpoons EP + \text{Arg}$$

where E is free enzyme and EP is a phosphorylated enzyme. In this case, using radioactive substrates labelled with ^{14}C [U-^{14}C]arginine and [^{14}C]ADP) we should observe incorporation of radioactivity in ATP or in arginine phosphate. We shall consider these two reactions successively.

(1) ATP–ADP exchange

(A) Reaction catalysed by arginine kinase. (a) Characterisation of this reaction.

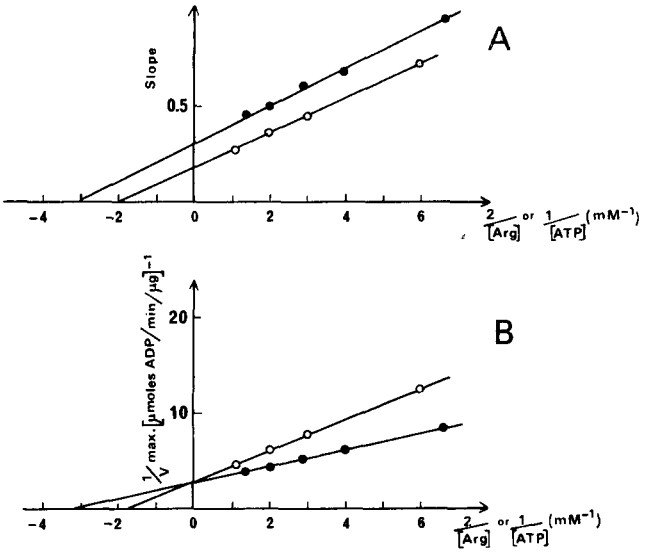


Fig. 2. Replots of slopes and intercepts (data on Fig. 1) as a function of the reciprocal of the arginine or ATP–Mg^{2+} concentrations. A. Slope *versus* 2/[arginine] (●—●) or 1/[ATP–Mg^{2+}] (○—○). B. $1/V$ *versus* 2/[arginine] (●—●) or 1/[ATP–Mg^{2+}] (○ - -○).

Fig. 3A shows the incorporation of radioactivity in ATP *versus* time. The maximum of ATP labelling occurs at the isotopic equilibrium between substrates. In this case, experimental data can be plotted according to $\log x_e/(x_e - x) = f(t)$ as shown in Fig. 3B. We can also observe that the exchange rate is proportional to enzyme concentration (Fig. 4A).

(b) pH and temperature effect. The radioactivity incorporation in ATP *versus* time for two temperatures (20 and 30°) is illustrated in Fig. 3A; the data point out the increase in the exchange rate with temperature.

The pH effect can be tested by the determination of exchange rates for several pH's. When $\log k$ is plotted against pH (ref. 29), the data obtained show a single pK with a value of about 6.7 in the pH range 6.5–8.0 (Fig. 4B).

(c) ADP concentration effect. Study of exchange rates as a function of ADP concentration, using a high ATP concentration, permits determination of the maximum exchange rate and the ADP dissociation constant [30] (Fig. 5A). In these experiments the concentration of free Mg^{2+} is maintained constant at 1 mM (ref. 31). The values so obtained are $K_{iADP} = 0.17$ mM and $v_{max} = 0.2$ nmole/min per μg.

(d) Comparison of exchange rates between partial reaction and substrate equilibrium reaction. The equilibrium exchange reaction is tested in the experimental conditions described in Table I. The substrate equilibrium constant is $K_{eq} = 0.18$. The exchange rate is 29 nmoles/min per μg. It is about two orders of magnitude faster than the partial exchange rate under the same conditions ($v = 0.17$ nmole/min per μg)

(e) Nucleotide diphosphokinase reaction. To eliminate the possibility of an effect from contaminating enzymes accounting for an exchange reaction, we study the following transfer reaction between nucleotides:

$$ADP + GTP \underset{Mg^{2+}}{\rightleftharpoons} ATP + GDP$$

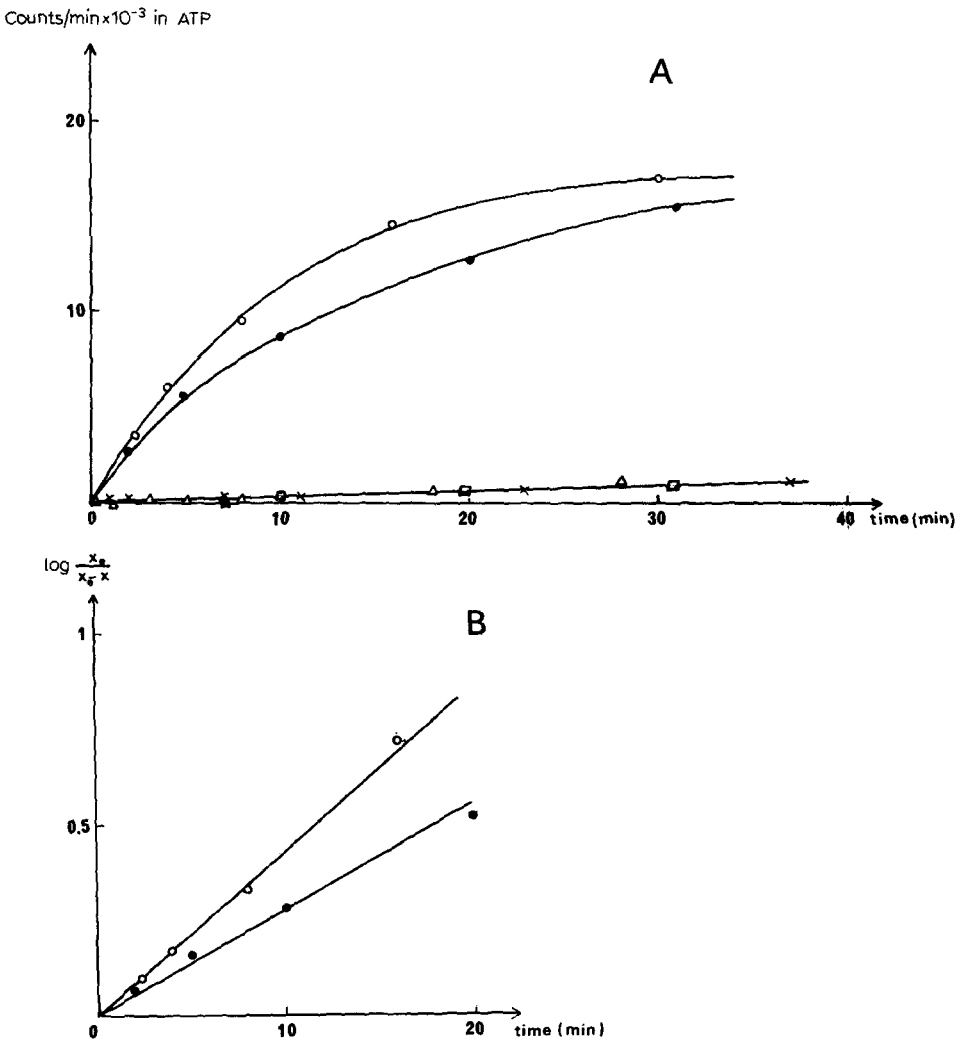


Fig. 3. ATP–ADP partial exchange reaction. A. Rate of [^{14}C]ATP formation from [^{14}C]ADP *versus* time. The reaction mixture contained in a final volume of 0.5 ml: 0.05 M Tris–acetate buffer (pH 8.0), 40 μg enzyme, 0.5 mM ATP, 1 mM magnesium acetate and 0.25 μC [^{14}C]ADP. The rate of [^{14}C]ATP formation was measured on 50-μl aliquots. Reaction catalysed by: ○—○, arginine kinase at 30°; ●—●, arginine kinase at 20°; □—□, partial exchange reaction essayed at 20° on arginine kinase with GTP and ADP as substrates (experimental conditions are described in METHODS); ×—×, creatine kinase at 20°; △—△, taurocyamine kinase at 20°. B. Determination of ATP–ADP exchange rate, catalysed by arginine kinase, by plotting log $x_e(x_e - x)$ *versus* time: ○—○, at 30°; ●—●, at 20°.

Under the experimental conditions described above, we cannot observe any ^{14}C incorporation into ATP. Thus, the partial exchange observed with arginine kinase cannot be explained by such a specific dinucleotide phosphokinase reaction (Fig. 3A).

(B) Studies on other phosphogen kinases. Similar partial exchange studies between [^{14}C]ADP and unlabelled ATP are performed under the same conditions and at

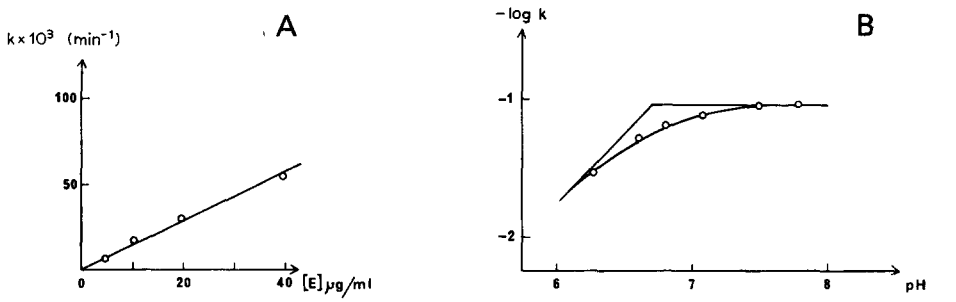
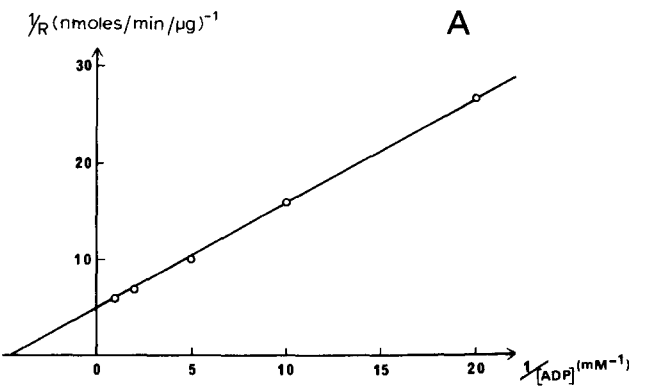


Fig. 4. ATP–ADP partial exchange reaction catalysed by arginine kinase. A. Effect of protein concentration on exchange rate constant. B. Effect of pH on exchange rate constant. Data were plotted according to DIXON[29]. Experimental conditions are described on Fig. 3.



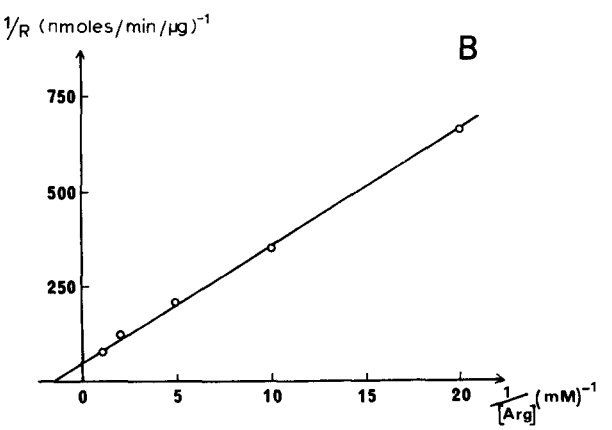


Fig. 5. Effect of substrate concentrations on partial exchange reaction catalysed by arginine kinase. A. Effect of ADP concentration on ATP–ADP exchange reaction. The reaction mixture, at 25°, contained in 1 ml: 0.05 M Tris–acetate buffer (pH 8.0), 80 µg enzyme, 5 mM ATP, several ADP concentrations (between 0.05 mM and 1.00 mM). The free [Mg^{2+}] is fixed at 1 mM. Reaction was started by addition of 0.5 µC [^{14}C]ADP. B. Effect of arginine concentrations on arginine phosphate–arginine exchange reaction. The reaction mixture at 25° contained in 0.5 ml: 0.05 M Tris–acetate buffer (pH 7.00), 92 µg enzyme, 1 mM arginine phosphate, several arginine concentrations (between 0.05 and 1.00 mM) and 0.5 µC [U-^{14}C] arginine.

TABLE I

EQUILIBRIUM ISOTOPIC EXCHANGE RATE

Experimental conditions: the reaction mixture contained in 0.1 M Tris–acetate buffer (pH 8.0, temp. 25°): 10 μg/ml enzyme and substrates at concentrations reported above. In assay No. II, initial concentrations of substrates were: 2.5 mM ATP, 0.5 mM ADP, 0.5 mM arginine and 3.5 mM Mg^{2+}. The exchange reaction is performed under experimental conditions of Fig. 3.

Assay No.	*Concentrations at equilibrium (mM)*							K_{eq}*	*ATP–ADP exchange rate (nmoles/min per μg)*
	[ATP]	*[ATP–Mg^{2+}]***	*[ADP]*	*[ADP–Mg^{2+}]***	*[Arg]*	*[Arg~P]*	*Total [Mg^{2+}]*		
I	2.28	2.25	0.72	0.50	0	0	3.5	—	0.17
II	2.28	2.25	0.72	0.50	0.28	0.22	3.5	0.18	29

* $K_{eq} = \dfrac{[\text{ADP–Mg}^{2+}]\,[\text{Arg}\sim\text{P}]}{[\text{ATP–Mg}^{2+}]\,[\text{Arg}]}$.

** Calculated from dissociation constants.

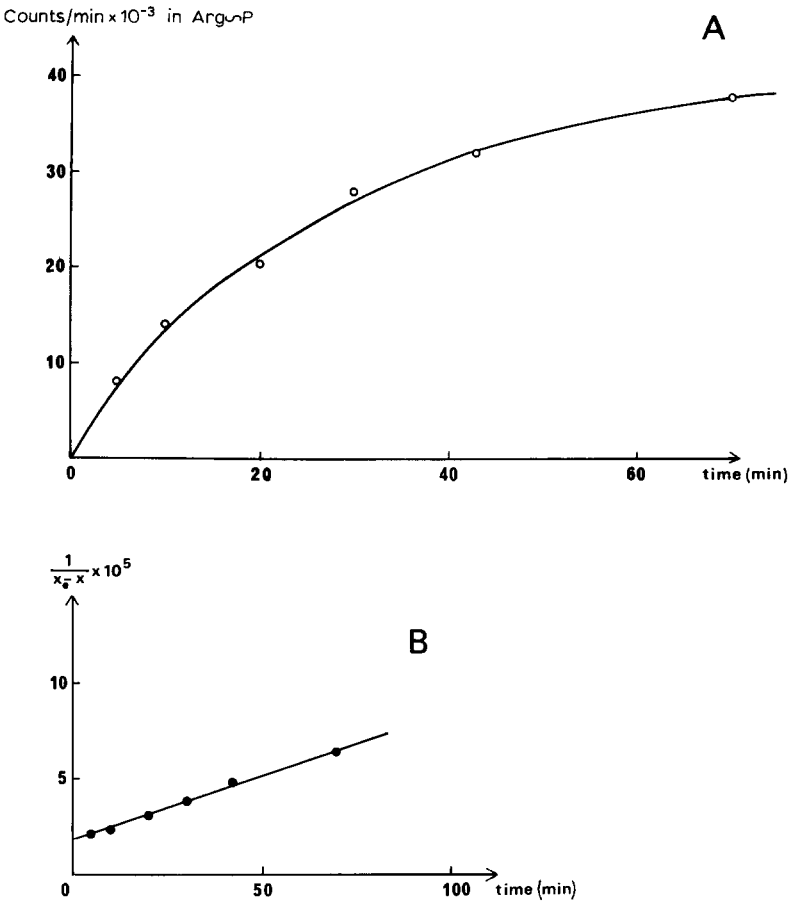


Fig. 6. Arginine phosphate–arginine partial exchange reaction catalysed by arginine kinase. A. Rate of [^{14}C]arginine phosphate formation from [U-^{14}C]arginine *versus* time. The reaction mixture contained in a 0.5 ml final volume: 0.05 M Tris–acetate buffer (pH 7.0), 92 μg enzyme, 1 mM arginine phosphate, 0.5 μC [^{14}C]arginine. The rate of [^{14}C]arginine phosphate formation was measued on 80-μl aliquots. B. Determination of exchange rate by plotting $1/x_e - x$) *versus* time.

the optimum pH for the transphosphorylation reactions with taurocyamine kinase and creatine kinase at several enzyme concentrations (20–90 μg). Fig. 3A illustrates one of these experiments and shows that the observed exchange reaction is insignificant as compared with that of arginine kinase.

(2) Arginine phosphate–arginine exchange reaction

(A) Characterisation. We have further demonstrated that a partial exchange reaction between arginine phosphate and arginine is catalysed by arginine kinase (Fig. 6). Under our experimental conditions the exchange rate is slower than that observed above in the ATP–ADP exchange reaction, because of the non-saturated concentration of arginine phosphate. A high arginine phosphate concentration, indeed, inhibits the transphosphorylation reaction[2]. On the other hand, we can also show that this rate is proportional to enzyme concentration.

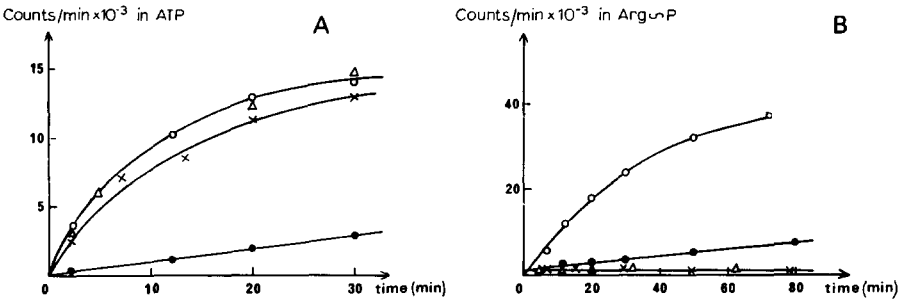


Fig. 7. Partial exchange reaction catalyzed by specifically modified arginine kinase. Experimental conditions described in METHODS. A. ATP–ADP exchange reaction. B. Arginine phosphate–arginine exchange reaction. ○—○, native enzyme; △—△, dansylated enzyme; ×—×, *S*-carboxymethylated enzyme; ●—●, histidyl modified enzyme.

(B) Effect of arginine concentration. If we study the partial exchange rate for various arginine concentrations in the 0.05–1.00 mM range, we can plot our data according to LINEWEAVER AND BURK[32] (Fig. 5B). The dissociation constant determined by extrapolation of the curve is $K_{iArg} = 0.7$ mM and the corresponding v_{max} in the presence of 1 mM arginine phosphate is 0.025 nmole/min per μg.

TABLE II

PARTIAL EXCHANGE REACTION RATES ON MODIFIED ARGININE KINASE

Chemical modification		*Inhibition (%)*	*Exchange rate (%)*	
Modified residue	*Amount of labelling (residues per mole of protein)*		*ATP–ADP*	*Arg–Arg ~ P*
Native enzyme	0	0	100	100
Cysteinyl	2	100	65	0
Histidyl	1.5	95	6	8
Lysyl	1.5	100	100	0

(III) Chemically modified arginine kinase

We can extend the latter exchange method for studying enzymes modified at cysteine, lysine and histidine residues which have been reported to be essential for the whole transphosphorylation reaction[9,10,36].

(1) S-Carboxymethyl arginine kinase

As shown in Fig. 7, a 100% inhibited arginine kinase, two sulphydryl groups of which are carboxymethylated (see MATERIALS AND METHODS), is able to catalyse the partial exchange ATP–ADP reaction, but at a lower rate (65% of the rate observed with the native enzyme). In contrast, the arginine phosphate–arginine exchange reaction does not occur (Table II).

(2) Lysine modified arginine kinase

The 100% inactivated dansylated enzyme, possessing all its sulphydryl groups, has a behaviour similar to the latter modified enzyme (Fig. 7). Whereas dansylation

up to 1.5 lysyl residues does not affect the ATP–ADP exchange reaction, it abolishes the arginine phosphate–arginine exchange (Table II).

(3) Histidine modified arginine kinase

Arginine kinase 95% inhibited after carbethoxylation of 1.5–1.8 histidyl residues exhibits no partial exchange reactions (Fig. 7, Table II).

DISCUSSION

The results reported above together with those of MORRISON[2] concerning initial velocity experiments on arginine kinase from several sources appear to be consistent with a mechanism of sequential type. This reaction may proceed via the interconversions of ternary complexes, which would be the slower steps. So this mechanism would be a common feature of the class of phosphagen kinases. Arginine kinase is the only enzyme which possesses a mechanism including a partial exchange reaction via binary complexes, in contrast to other kinases studied (creatine and taurocyamine kinases). The exchange rate of the partial reaction, however, is slower than the equilibrium exchange rate. That this partial exchange reaction exists and is specific with arginine kinase is supported by the following findings:

The rate varies with protein concentration. In the case of the ATP–ADP exchange, the rate is also influenced by temperature, and the presence of Mg^{2+} is necessary. The possibility of influence by a contaminating enzyme such as nucleotide diphosphokinase is excluded; this latter reaction, indeed, does not occur with the true substrate GDP (ref. 33). Of further interest is the observation that the partial exchange reaction occurred with the two pairs of substrates; this result underlines again the specificity of this reaction. Furthermore, exchange reaction studies lead to customary values for ADP and arginine dissociation constants[22,34].

On an other hand, the effect of pH on the ATP–ADP exchange rate reveals a pK with a value of about 6.7; this value suggests the participation of a histidyl residue. A histidyl residue is, indeed, involved in the active site[7,10] and its role was previously demonstrated to be connected with the catalytic process[6]. The value of this pK is lower than that related to the whole transphosphorylation (unpublished results); the data suggest that the formation of ternary complexes could modify the pK of essential histidine, thenceforth ternary complexes would be more efficient. The binding of the second substrate would enhance the rate of isotopic exchange according to BRIDGER *et al.*[30], so histidine would have an efficient role.

The second hypothesis formulated by MORRISON[2] would be that the transfer of HPO_3^- occurs by a two-reaction flux, the ping-pong flux being very slow.

The study of the partial exchange reaction with chemically modified enzymes may be, indeed, a valuable tool for understanding the relationship between the nature and the role of different essential amino acid residues in the active site.

If we modify the essential cysteinyl residue included in the peptide, the amino acid sequence of which was determined[35] and which was probably involved in the L-arginine binding[22,36], we do not observe the arginine phosphate–arginine exchange reaction, although ATP–ADP exchange still occurs. These data provide further evidence that the essential cysteinyl residue is in fact involved in the arginine binding process, but they do not exclude an additional role in catalysis.

Previous studies[6] on lysine modified arginine kinase carried out by means of

difference spectrophotometry have shown that arginine binding does not occur, suggesting that the ε-amino group would be near the essential cysteinyl residue. In the same way dansylation may prevent the isotopic exchange between guanidine substrates. Furthermore, although lysine seems to be involved in protonation of ADP during the whole transphosphorylation reaction, it does not necessarily appear critical in the case of the partial reaction. Finally the most significant result points out the central role of the histidyl residue in the catalytic process: although all substrates bind to the specifically histidyl modified and inhibited[6] enzyme, neither partial exchange nor transphosphorylation reaction occur.

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REFERENCES

1 R. VIRDEN, D. C. WATTS AND E. BALDWIN, *Biochem. J.*, 94 (1965) 536.
2 E. SMITH AND J. F. MORRISON, *J. Biol. Chem.*, 244 (1969) 4224.
3 L. NODA, T. NIHEI AND M. F. MORALES, *J. Biol. Chem.*, 235 (1960) 2830.
4 W. J. O'SULLIVAN, R. VIRDEN AND S. BLETHEN, *Eur. J. Biochem.*, 8 (1969) 562.
5 W. J. O'SULLIVAN AND M. COHN, *J. Biol. Chem.*, 241 (1966) 3104.
6 C. ROUSTAN, L. A. PRADEL, R. KASSAB, A. FATTOUM AND N. V. THOAI, *Biochim. Biophys. Acta*, 206 (1970) 369.
7 R. VIRDEN AND D. C. WATTS, *Biochem. J.*, 99 (1966) 162.
8 R. KASSAB, L. A. PRADEL, E. DER TERROSSIAN AND N. V. THOAI, *Biochim. Biophys. Acta*, 132 (1967) 347.
9 R. KASSAB, C. ROUSTAN AND L. A. PRADEL, *Biochim. Biophys. Acta*, 167 (1968) 308.
10 L. A. PRADEL AND R. KASSAB, *Biochim. Biophys. Acta*, 167 (1968) 317.
11 E. DER TERROSSIAN, R. KASSAB, L. A. PRADEL AND N. V. THOAI, *Biochim. Biophys. Acta*, 122 (1966) 462.
12 N. V. THOAI, L. A. PRADEL AND R. KASSAB, in H. TABOR AND C. W. TABOR, *Methods in Enzymology*, Vol. XVIIA, Academic Press, New York, 1970, p. 1002.
13 T. A. MAHOWALD, E. R. NOLTMANN AND S. A. KUBY, *J. Biol. Chem.*, 237 (1962) 1535.
14 N. V. THIEM, N. V. THOAI AND J. ROCHE, *Bull. Soc. Chim. Biol.*, 44 (1962) 285.
15 R. M. BOCK, W. S. LING, S. A. MORELL AND S. H. LIPTON, *Arch. Biochem. Biophys.*, 62 (1956) 253.
16 S. A. KUBY, L. NODA AND H. A. LARDY, *J. Biol. Chem.*, 209 (1954) 191.
17 C. ORIOL-AUDIT, M. F. LANDON, Y. ROBIN AND N. V. THOAI, *Biochim. Biophys. Acta*, 188 (1969) 132.
18 L. A. PRADEL, R. KASSAB, F. REGNOUF AND N. V. THOAI, *Biochim. Biophys. Acta*, 89 (1964) 255.
19 N. V. THOAI, R. KASSAB AND L. A. PRADEL, *Biochim. Biophys. Acta*, 110 (1965) 532.
20 S. A. KUBY, L. NODA AND H. A. LARDY, *J. Biol. Chem.*, 209 (1954) 203.
21 R. KASSAB, L. A. PRADEL AND N. V. THOAI, *Biochim. Biophys. Acta*, 99 (1965) 397.
22 C. ROUSTAN, R. KASSAB, L. A. PRADEL AND N. V. THOAI, *Biochim. Biophys. Acta*, 167 (1968) 326.
23 G. ELLMAN, *Arch. Biochem. Biophys.*, 82 (1959) 70.
24 B. S. HARTLEY AND V. MASSEY, *Biochim. Biophys. Acta*, 21 (1956) 58.
25 P. ELODI, in N. V. THOAI AND J. ROCHE, *Biochemical Evolution and Homologous Enzymes*, Gordon and Breach, New York, 1968, p. 105.
26 N. V. THIEM, G. LACOMBE AND N. V. THOAI, *Biochim. Biophys. Acta*, in the press.
27 J. R. FLORINI AND C. S. WESTLING, *Biochim. Biophys. Acta*, 25 (1957) 557.
28 W. W. CLELAND, *Biochim. Biophys. Acta*, 67 (1963) 104.
29 M. DIXON, *Biochem. J.*, 55 (1953) 161.
30 W. A. BRIDGER, W. A. MILLEN AND P. D. BOYER, *Biochemistry*, 7 (1968) 3608.

31 J. F. MORRISON, W. J. O'SULLIVAN AND A. G. OGSTON, *Biochim. Biophys. Acta*, 52 (1961) 82.
32 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
33 R. L. RATLIFF, R. H. WEAVER, H. A. LARDY AND S. A. KUBY, *J. Biol. Chem.*, 239 (1964) 301.
34 M. L. UHR, F. MARCUS AND J. F. MORRISON, *J. Biol. Chem.*, 241 (1966) 5428.
35 E. DER TERROSSIAN, L. A. PRADEL, R. KASSAB AND N. V. THOAI, *Eur. J. Biochem.*, 11 (1969) 482.
36 C. ROUSTAN, E. DER TERROSSIAN AND L. A. PRADEL, *Eur. J. Biochem.*, 17 (1970) 467.