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Electron Transport Coupled to Quasi-Arsenylation in Isolated Chloroplasts

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Abstract

Ferricyanide reduction in isolated chloroplasts has been known to be inhibited by ATP. AMP and arsenate restored this ATP-inhibited ferricyanide-reducing acitivity. The nature of restoration caused by an ATP-AMP-arsenate system (termed here quasi-arsenylation) was found to be quite similar to the nature of restoration coupled to phosphorylation or arsenylation. The optimum pH for both arsenylation and quasi-arsenylation was around 8.3. From a preliminary analysis of the restoration process by quasi-arsenylation, the apparent *Km* values for ATP (at AMP = ∞) and AMP (at ATP = ∞) were estimated to be around 20 and 6 μM, respectively.

The mechanism of restoration coupled to quasi-arsenylation is discussed in relation to the chloroplast coupling factor (CF_1) which can bind either two ADP molecules or one each of ATP and AMP molecules.

Introduction

As far as the effect of adenine nucleotides is concerned, in isolated chloroplasts, there are apparently two non-cyclic electron transport systems [1-3], one of which is regulated by adenine nucleotides (phosphorylation coupled) and the other independent of them (basal [2]). In the preceding paper [1], we presented a possible explanation of the mechanism of electron-transport regulation by adenine nucleotides.

The electron transport regulated by adenine nucleotides involves two processes which take place sequentially with an increase in the nucleotide concentration [1]. In the first process, electron transport is gradually inhibited, probably due to a decrease in the leakage of protons

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accumulated inside the thylakoid sacs [4, 5], presumably through a 1:1 binding of ADP or ATP (but not AMP) by CF_1 at the inhibition site [1]. In the second process, the once-inhibited electron transport activity is apparently restored in the presence of P*i* (or A*s*, arsenate), due to the turnover of phosphorylation (or arsenylation) which requires translocation of protons [6], when the CF_1 with which the first ADP has been associated binds the second ADP (but not ATP) in a 1:1 ratio at the coupling site [1].

ATP, even in the presence of P*i* (or A*s*) causes only inhibition but not restoration. AMP, regardless of the presence of P*i* (or A*s*), causes neither inhibition nor restoration.

However, we found that the simultaneous presence of ATP and AMP in the presence of A*s* (but not P*i*) induced an apparent restoration (stimulation) of electron transport from the level inhibited by ATP. We called this "quasi-arsenylation" and discuss a possible reaction mechanism in relation to the mechanism of arsenylation (and phosphorylation).

Experimental Procedures

Chloroplasts were prepared from market spinach leaves with a 0.5 M choline medium [7, 8] by a procedure described previously [7]. Fecy (ferricyanide)-reducing activity was measured in a medium composed of 0.1 M sucrose, 5 mM $MgCl_2$, 10 mM tricine at pH 8.3 and 600 μM Fecy at 15°C by following the absorbance decrease at 420 nm after actinic illumination (5×10^4 lux, white light) for a few minutes. Chloroplasts used were 20 μg/ml in the chlorophyll concentration. Nucleotides (from Sigma Chemical Co.), A*s*, phlorizin and gramicidin S were added to the reaction mixture as required. In experiments of the pH dependence of Fecy reduction, a mixed buffer composed of citrate, piperazine-N-N′-bis(2-ethane sulfonic acid), tricine and sarcosine (10 mM each) was used instead of a single tricine buffer.

Results and Discussion

Figure 1 shows the activity profiles of Fecy reduction observed under various conditions. As reported in the preceding paper [1], with an increase in ATP (or ADP) concentration, Fecy-reducing activity gradually decreased and reached the lowest level at an ATP (or ADP) concentration of around 10 μM. This lowest level shifted to some higher level if A*s* (or P*i*) was absent [1]. AMP (as reported in ref. [1]) or AMP with A*ss* (or P*i*) did not inhibit Fecy reduction at any concentration up to 100 μM. When the ADP concentration further increased in the

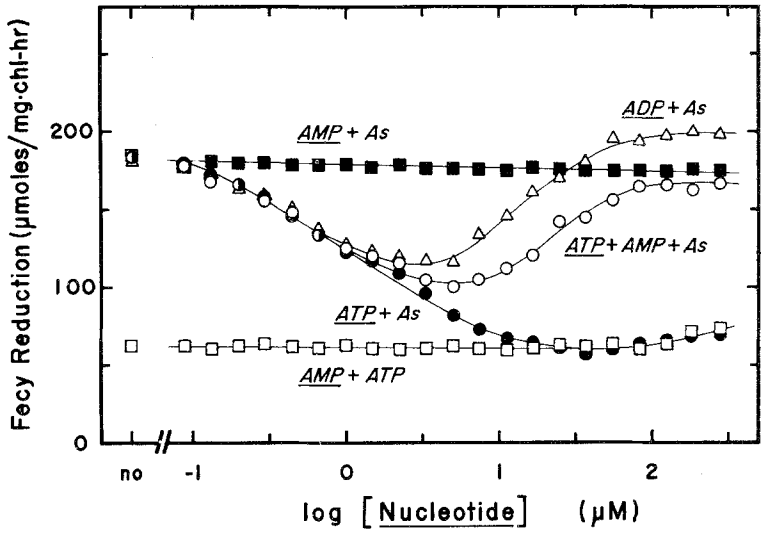

Figure 1. Activity-concentration profiles of Fecy reduction coupled to arsenylation or quasi-arsenylation. The concentration of the underlined nucleotide was changed. The fixed concentrations of As, AMP and ATP were 1 mM, 100 μM and 100 μM, respectively. Fecy reduction was measured at pH 8.3 and 15°C.

presence of As (or Pi, e.g. at 1 mM), restoration of the once-inhibited Fecy-reducing activity took place in parallel with arsenylation (or phosphorylation) as reported.

We found that when AMP and As (but not Pi; discussed elsewhere) were simultaneously present, an apparent restoration occurred with an increase in the ATP concentration. The activity profile of Fecy reduction coupled to this ATP-AMP-As system (termed here "quasi-arsenylation") resembled that coupled to arsenylation (the ADP-As system; or phosphorylation, the ADP-Pi system), except for the extent of maximum restoration. If As was absent, the activity level inhibited by ATP was not restored by an increase in the AMP concentration.

Figure 2A shows the pH dependence of Fecy-reducing activities measured in the presence of 200 μM ADP and 1 mM As ($Fecy_{ADP,As}$), 100 μM ATP, 100 μM AMP and 1 mM As ($Fecy_{ATP,AMP,As}$), and 100 μM ATP and 1 mM As ($Fecy_{ATP,As}$). Figure 2B shows the extent of restoration due to either arsenylation ($\Delta Fecy'_{restored} = Fecy_{ADP,As} - Fecy_{ATP,As}$) or quasi-arsenylation ($\Delta Fecy'_{restored} = Fecy_{ATP,AMP,As} - Fecy_{ATP,As}$). Both $\Delta Fecy_{restored}$ and $\Delta Fecy_{restored}$ showed similar pH dependence curves with the same pH optimum of about 8.3, which is slightly lower than the value (8.5) for the restoration due to phosphorylation [1].

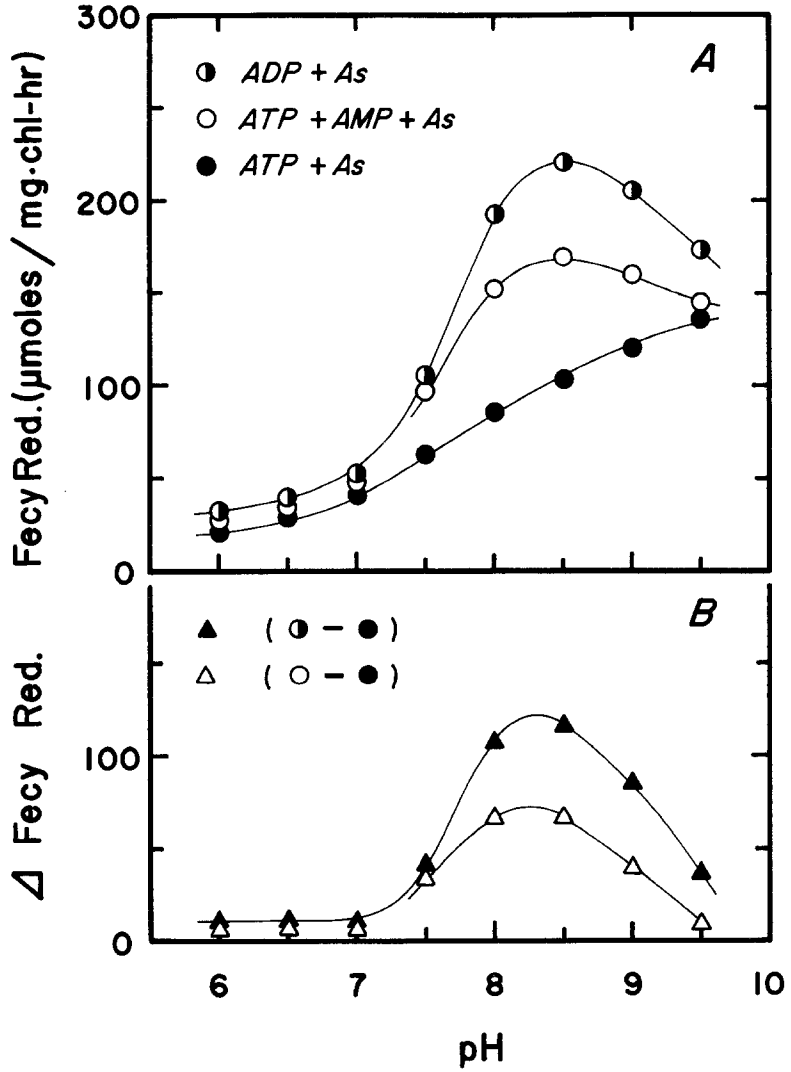

Figure 2. (A) pH Dependence of the Fecy-reducing activities. Fecy reduction was measured at 15° C in the presence of 200 μM ADP and 1 mM As (◑, Fecy$_{ADP,As}$), or 100 μM ATP, 100 μM AMP and 1 mM As (○, Fecy$_{ATP,AMP,As}$), or 100 μM ATP and 1 mM As (●, Fecy$_{ATP,As}$). (B) pH Dependence of the difference in the Fecy-reducing activities shown in (A).

Figure 3 shows the effects of phlorizin and gramicidin S on Fecy-reducing activities coupled to either arsenylation or quasi-arsenylation. Phlorizin did not affect the inhibition by ADP or ATP

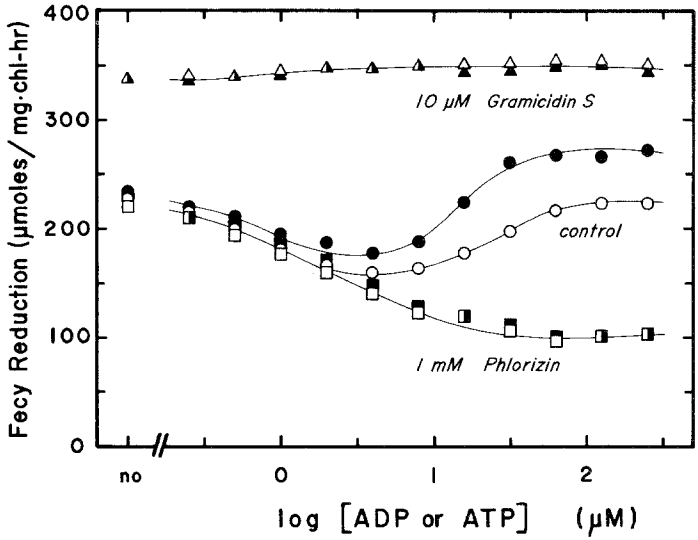

Figure 3. Effects of phlorizin and gramicidin S on Fecy reduction coupled to either arsenylation or quasi-arsenylation. Fecy reduction was measured at pH 8.3 and 15°C under the condition of arsenylation (200 μM ADP + 1 mM As; closed symbols) or quasi-arsenylation (100 μM ATP + 100 μM AMP+ 1 mM As; open symbols) in the presence of 1 mM phlorizin (squares) or 10 μM gramicidin S (triangles) or in their absence (control, circles).

(with AMP) but depressed the restoration coupled to arsenylation or quasi-arsenylation. The effect of this energy transfer inhibitor was quite similar to that observed for Fecy-reducing activity coupled to phosphorylation [9]. Gramicidin S depressed the extent of both inhibition and restoration and raised the lowest (zero) level of the activity coupled to arsenylation or quasi-arsenylation. The effect of this uncoupler was also similar to that on Fecy-reducing activity coupled to phosphorylation [9].

Figure 4A compares the As requirements for arsenylation and quasi-arsenylation. For the reaction coupled to arsenylation or quasi-arsenylation, the level at As = 0 was approximated as the zero level and then Lineweaver-Burk plots for the reactions were obtained as in Fig. 4B. In both cases, the *Km* value for As was about 230 μM, while the V_{max} values differed from each other. The absolute V_{max} values depended on the chloroplast preparations, but the value for arsenylation was always not smaller than that for quasi-arsenylation.

These results strongly imply that the regulation of electron transport by quasi-arsenylation involves a mechanism common to the regulation by phosphorylation or arsenylation, where CF_1 plays an essential role [10, 11]. Results of Roy and Moudrianakis [12] and Forti *et al.* [13]

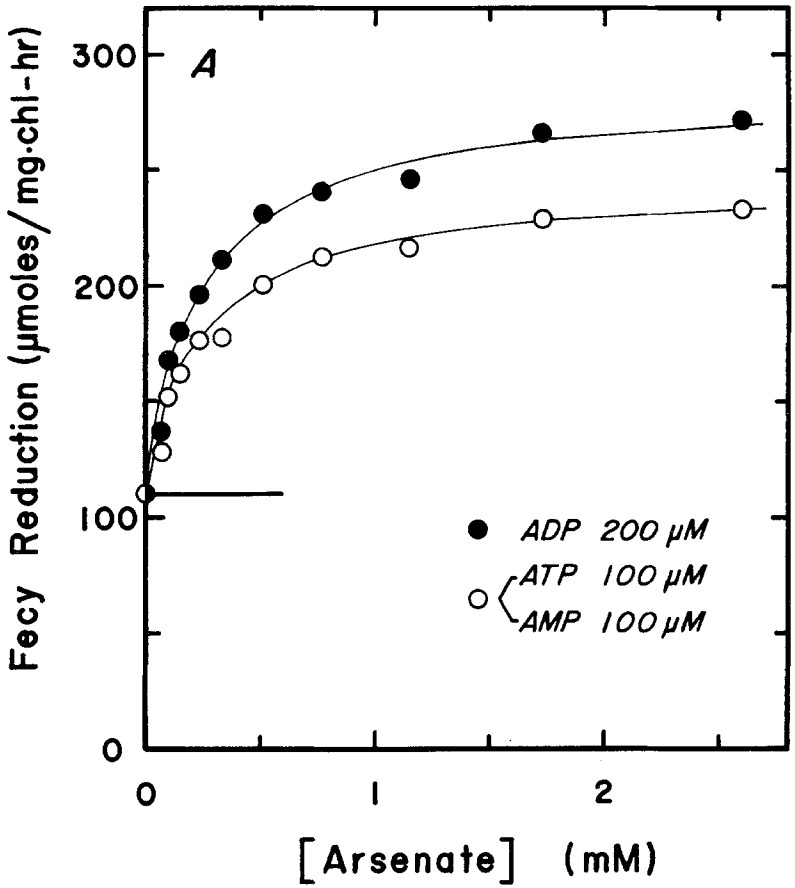

Figure 4. (A) Arsenate concentration dependence of Fecy reduction coupled to arsenylation or quasi-arsenylation. Fecy reduction was measured at pH 8.3 and 15°C in the presence of either 200 μM ADP (●; arsenylation-coupled) or 100 μM ATP and 100 μM AMP (○, quasi-arsenylation-coupled) at various concentrations of As. (B) Lineweaver-Burk plot of the As-dependent Fecy reduction (above the level of As = 0) shown in (A).

indicate that binding of two ADP molecules to one CF_1 is a key step in photophosphorylation. Girault *et al.* [14] reported that purified CF_1 binds ADP stepwise with an increase in the ADP concentration, and determined the association constants for the first and second ADP binding by CF_1. Based on this physicochemical data [14], we showed [1] that Fecy-reducing activity was inhibited, through a conformation change [4, 5] in CF_1, as CF_1 bound the first ADP at the inhibition site and then restored when phosphorylation (or arsenylation) took place as

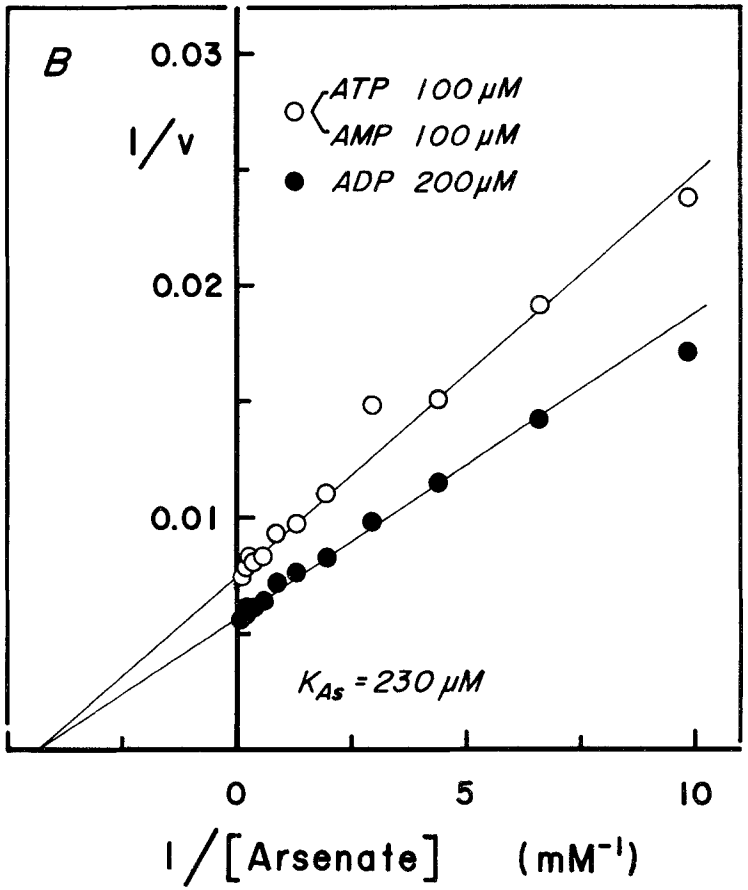

Fig. 4(B).

the CF_1 bound the second ADP at the coupling site at higher ADP concentrations in the presence of *Pi* (or *As*). Since the inhibition site had the same affinity for ATP [1, 14] as for ADP, the iibition was observed as CF_1 bound the first ATP, but no further change in Fecy-reducing activity was observed even when the ATP concentration increased up to 1 mM (except for the effect of contaminating ADP in ATP) in the presence of *Pi* or *As*. AMP, regardless of the presence of *Pi* or *As*, did not show any regulation of electron transport when added externally. In other words, the binding of AMP by CF_1 could not be detected through electron transport activity. Otherwise, AMP could not induce a conformation change even though it bound to the inhibition site.

As Roy and Moudrianakis proposed [12], if two ADP molecules are bound by CF_1 (to form E_{ADP}^{ADP}), and become the substrates for the

succeeding transphosphorylation to produce ATP and AMP, these nucleotides are, at least for a while, bound by CF_1 (to form E^{AMP}_{ATP}). Then, it is likely that when the inhibition site is occupied by ATP, a conformation change in CF_1 is induced, then the coupling site becomes ready to bind AMP. If this is the case, the binding of AMP to E_{ATP} could be analyzed as well as that of ADP to E_{ADP} had been [1].

Figure 5 represents the activity profiles of Fecy reduction *vs.* AMP concentration, at four ATP concentrations which were sufficient to cause maximum inhibition at AMP = 0. As the AMP concentration

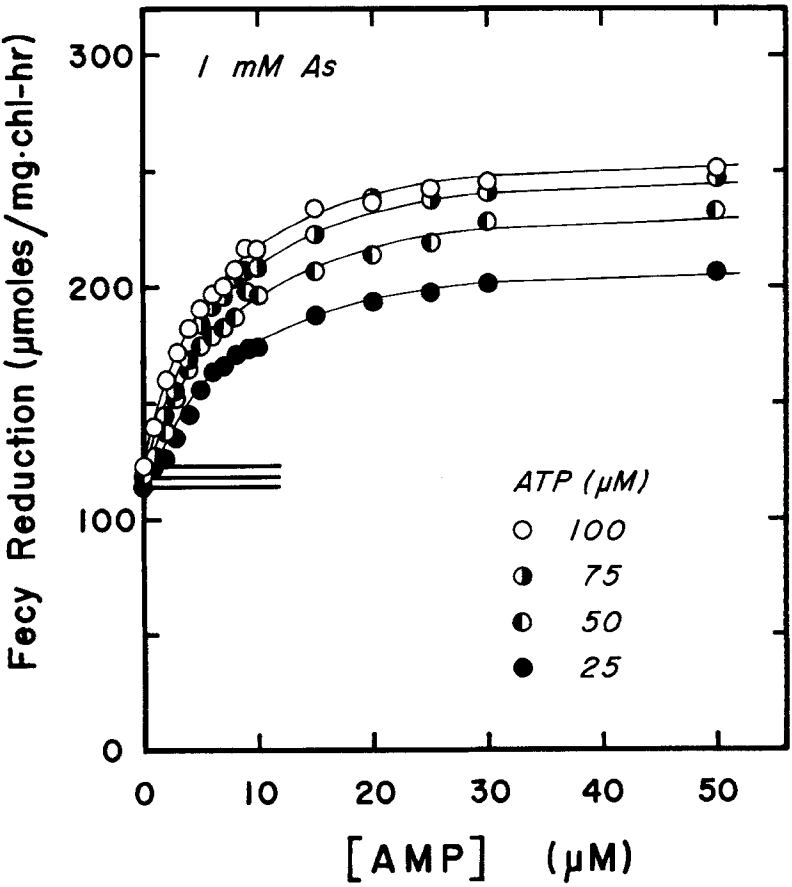

Figure 5. Effects of ATP and AMP on the quasi-arsenylation-coupled Fecy reduction. Fecy reduction was measured at pH 8.3 and 15°C in the presence of 1 mM As, ATP as given in the figure and various amounts of AMP. The AMP-dependent Fecy reduction (above the level at AMP = 0) was preliminarily used for analysis of the reaction.

increased in the presence of As, restoration of the ATP-inhibited Fecy reduction took place. The extent of restoration depended on the concentration of ATP present simultaneously. The mechanism of this two substrate reaction and the effect of inhibitors on the reaction are under investigation. A preliminary analysis using Lineweaver-Burk-type plots of the results shown in Fig. 5 (zero level = level at AMP = 0) gave the apparent *Km* values of approximately 20 μM for ATP (at AMP = ∞) and 6 μM for AMP (at ATP = ∞). These values suggest that the binding of AMP by CF_1 at the coupling site is somewhat stronger than the binding of ADP (Km = 17 μM [1]). This may correspond to the observation [12] that there were some AMP molecules tightly bound by CF_1 and this bound AMP was not formed when AMP was simply added externally.

One possible and tentative explanation for the mechanism of restoration coupled to either arsenylation or quasi-arsenylation is based on the following assumptions. Whichever the inhibition site is occupied by ADP or ATP, AMP at the coupling site reacts with As in parallel with translocation of protons accumulated inside the thylakoid sacs. AMP·As thus formed on the coupling site is spontaneously hydrolyzed into As liberated and AMP still bound to the coupling site, not taking the next step of trans-arsenylation. Consequently, the electron transport functions to pump in protons in order to complement the amounts

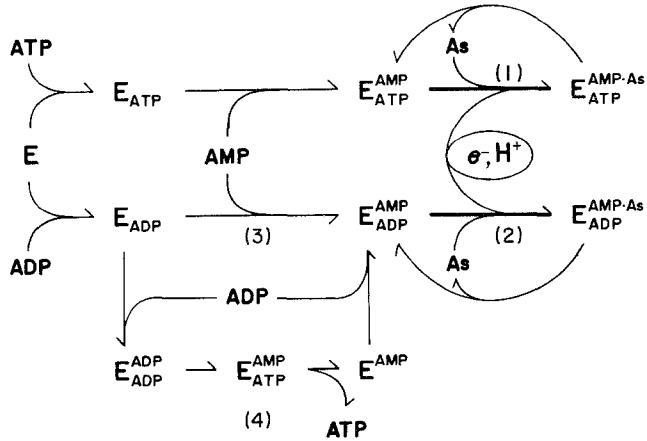

Figure 6. Tentative scheme for the mechanisms of arsenylation and quasi-arsenylation. E denotes CF_1. The upper side of E is regarded as the coupling site and the lower side as the inhibition site. e^-,H^+ represents the energized driving system for quasi-arsenylation (1), arsenylation (2) and phosphorylation (not shown). The pathway (4)-(2) is for the arsenylation (ADP-As) system and involves a part of the phosphorylation (if As is replaced by Pi) system. The pathway (3)-(2) is an explanation for the apparent stimulation of arsenylation-coupled Fecy reduction by AMP in a certain concentration range of ADP (Fig. 7).

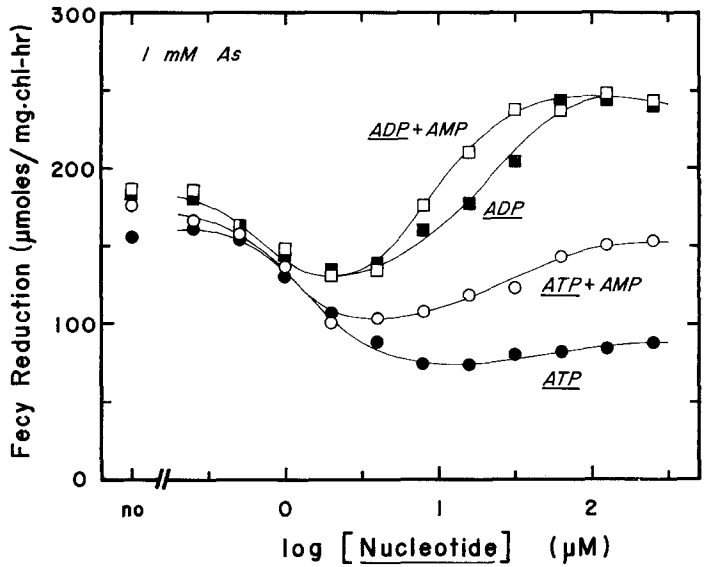

Figure 7. Effects of AMP on Fecy reduction in the presence of As and ADP or ATP. Fecy reduction was measured at pH 8.3 and 15°C in the presence of 1 mM As and 10 μM AMP and various amounts of either ADP or ATP.

translocated by arsenylation or quasiarsenylation, both of which are thus energy-dissipating (Fig. 6). This idea is supported by the fact that Fecy-reduction coupled to arsenylation was stimulated by the simultaneous presence of AMP (e.g. 10 μM) as shown in Fig. 7.

In case of quasi-phosphorylation, which has not been found to occur,* AMP at the coupling site would not be phosphorylated as the inhibition site is occupied by ATP, or even though AMP is phosphorylated the succeeding transphosphorylation from ADP thus formed to ATP at the inhibition site would not take place (further discussed elsewhere).

The difference between arsenylation and quasi-arsenylation would thus be attributable to the species of adenine nucleotides bound to the inhibition site. If ADP binds to the inhibition site, the reaction is called arsenylation. If ATP binds, we termed it here quasi-arsenylation.

Acknowledgments

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* Therefore, an adenylate kinase type reaction would not be involved in our reaction system, unless the kinase is activated only in the presence of Pi.

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