Fe^{III}-ion-Catalysed Non-enzymatic Transformation of ADP into ATP

Akira Kitani, Shu-ichi Tsunetsugu and Kazuo Sasaki *
Department of Applied Chemistry, Hiroshima University, Higashi-Hiroshima, 724 Japan

Non-enzymatic phosphorylation of ADP to ATP has been found to occur readily when Fe^{III} ion is present in aqueous solutions of ADP and acetyl phosphate. As long as the reaction temperature is low enough (25 °C or less), the phosphorylation of ADP to ATP proceeds to about 20% conversion with 100% selectivity. At higher temperatures, the yield decreases owing to the hydrolysis of acetyl phosphate. Among several phosphoryl donors studied, acetyl phosphate was found to be the best phosphorylating reagent. Creatine phosphate also produced ATP but the efficiency was very low. The phosphorylation of AMP was unsuccessful, even with acetyl phosphate.

Non-enzymatic phosphorylation of adenosine has been one of the most attractive problems for chemists as well as biologists. Several pioneer works have been published, mostly in the last decade.[1-4] Hosseini *et al.*[4] reported that protonated macrocyclic polyamines, particularly [24]-N_6O_2, catalyse the solvolysis of acetyl phosphate (AcP) in Me_2SO–D_2O mixtures. When a phosphoryl acceptor, such as adenosine monophosphate (AMP) or diphosphate (ADP), is in the system a phosphoryl group is transferred to the acceptor to yield ADP and ATP, respectively. Certain divalent metal cations such as Ba^{2+} and Ca^{2+} can also catalyse the phosphoryl transfer from carbamyl phosphate to ADP.[2] Of these, Takeuchi[1] reported recently that ADP was converted into ATP when a solution of ADP was electrolysed in the presence of acetyl phosphate (AcP) with a particular titanium-based ceramic electrode. His work attracted our attention in several respects. First of all, it was the fundamental question of how and why the electrolysis, which is basically composed of electron transfer reactions, can be related to the phosphate transfer reaction. The present work has been attempted in order to answer this question.

Experimental

Materials and Chemicals.—The titanium-based ceramic (Toshiba Tungalloy, MK70X, 50 × 15.5 × 3.5 mm) was kindly supplied by Dr. Takeuchi of National Chemical Labs., Tsukuba. TiN and TiC were prepared in this laboratory by sputtering on an alumina plate surface in an argon atmosphere. Adenosine and adenosine phosphates (potassium salt for ADP and disodium salt for AMP) were purchased from Boehringer, and high-energy organic phosphates from Sigma Chemicals.

Electrolysis.—Electrolysis was carried out by controlling the anode potential in solutions having a similar composition to that specified by Takeuchi[1] (ADP, 2×10^{-3} mol dm^{-3}; acetyl phosphate, 4×10^{-3} mol dm^{-3}, in the stock solution containing 8 wt% KCl. The pH value of the solution was initially 4.7, which decreased to 3–4 after electrolysis).

Catalytic Reactions.—To initiate the reactions, given amounts of various metal salts were added to deaerated, aqueous solutions (10 cm^3) of ADP and organic phosphate. The solution was then agitated by vibration for several hours.

Product Analysis.—An HPLC unit was used for determining the concentration of adenosine phosphates. The eluent used was an equivolume mixture of acetonitrile and an aqueous solution of 0.3 mol dm^{-3} ammonium dihydrogen phosphate. In order to confirm that the product was really ATP, the enzymatic activity of the product was studied using the D-glucose test combination supplied by Boehringer.

Results and Discussion

We first tried to confirm the results reported by Takeuchi.[1] According to him, the nature of the anode material appeared to have a critical effect, and only the use of a ceramic electrode (titanium nitroxycarbide) was successful. Other materials, carbon, platinum and an alumina-based ceramic, were reported to be of no use. These observations were perfectly confirmed by our experiment, which was carried out under potentiostatic conditions (anode potential was fixed at 1.0 V *vs.* SCE). When the anode was of MK70X, which was used by Takeuchi, the conversion of ADP into ATP was observed after only 15 min of electrolysis, whereas with other electrodes no reaction was observed, even after 12 h or more of electrolysis. In addition to those materials reported by Takeuchi, we also tested Ti-carbide, and Ti-nitride as well as Ti–N–C ternary alloy as the anode material, but no ADP conversion was observed.

Two questions arise at this point: (*i*) is the anodic oxidation a necessary requirement for the reaction and (*ii*) why is ADP phosphorylated specifically at this particular electrode (MK70X)?

In order to answer these questions, we first studied the anodic behaviour of the ADP-containing solution by cyclic voltammetry, using MK70X as the working electrode. Although an appreciable oxidation current appeared in the first anodic trace of the cyclic voltammogram, the current diminished rapidly in the subsequent potential sweeps. The current cannot be regarded as being caused by any of the solution components, suggesting that the current should be attributed to some surface species of the ceramic anode. In fact, after 15 min of electrolysis with the MK70X electrode at 1.0 V SCE, the solution became white and a precipitate appeared, indicating that the electrode was corroded. We thus suspected that some of the corroded material might promote the phosphorylation.†

---

† Conclusive evidence for the irrelevance of the electrolysis in the ATP formation reaction was obtained by a control experiment using an iron electrode. When an iron wire was anodized at 0.8 V *vs.* SCE in an ADP solution for a short period (*ca.* 1 min.), passing a charge of 5 C, no ATP was detected immediately after the electrolysis. When the solution was left to stand for 1 h, however, ATP was detected at a concentration of 0.134×10^{-3} mol dm^{-3} and the concentration of iron dissolved was 1.22×10^{-3} mol dm^{-3}. Similar results were obtained with the MK70X electrode. No ATP was found in the solution immediately after electrolysis, but appeared (0.029×10^{-3} mol dm^{-3}) after 1 h, with dissolution of iron (0.22×10^{-3} mol dm^{-3}).

Table 1 Catalytic activity of Fe^{II} and Fe^{III} ions[a]

Catalyst	Concentration/ 10^{-3} mol dm^{-3}	Yield of ATP/ 10^{-3} mol dm^{-3}
$Fe_2(SO_4)_3$	0.48	0.42
$FeSO_4$	0.36	0.02

[a] Reaction was carried out in a non-buffered aqueous solution containing 2×10^{-3} mol dm^{-3} ADP and 4×10^{-3} mol dm^{-3} AcP for 5 h at 30 °C. In the case of Fe^{II}, an excess of hydroxylamine was added.

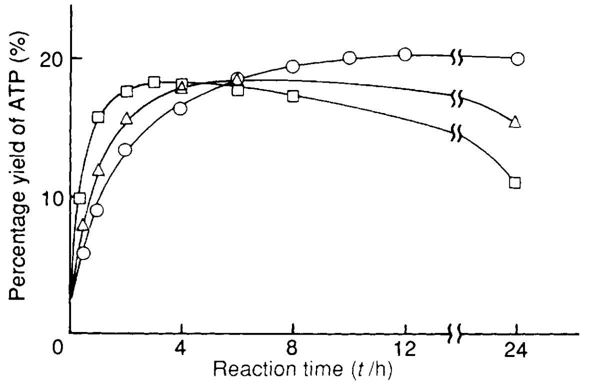

Fig. 1 The course of ATP formation with time at different temperatures: ○, 23 °C; △, 30 °C; □, 37 °C. Solution composition: 1×10^{-3} mol dm^{-3} ADP + 2×10^{-3} mol dm^{-3} AcP + 0.24×10^{-3} mol dm^{-3} $Fe_2(SO_4)_3$ in pure water.

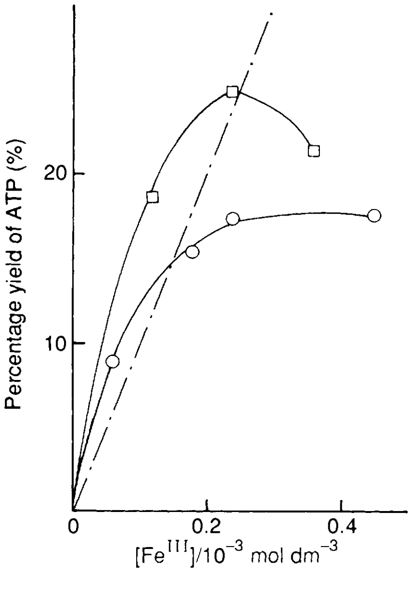

Fig. 2 Variation of ATP yield with increasing concentration of Fe^{III} ion for two different concentrations of acetyl phosphate (○, 2×10^{-3} mol dm^{-3}; □, 4×10^{-3} mol dm^{-3}). Broken line indicates the stoichiometric relationship between ATP and Fe^{III}. T = 30 °C, reaction time = 3 h.

The corrosion of MK70X was overlooked in Takeuchi's experiment.

In order to continue this investigation, we analysed the components of the ceramic by means of an energy dispersion X-ray microanalyser. The analysis indicated that, besides the three dominant components, N, C and O, several minor components such as Fe, Ni, Co, Mn, Zn and Ce are present in the ceramic. Accordingly, the effect of these elements on the phosphorylation reaction was studied for each element individually. A solution containing 2×10^{-3} mol dm^{-3} ADP, 4×10^{-3} mol dm^{-3} AcP, 8 wt.% KCl and 2–3 $\times 10^{-3}$ mol dm^{-3} of metal salt was prepared, and the mixture was agitated for several hours. The temperature was kept constant at 30 °C. The metal salts studied were $FeCl_2$, $NiCl_2$, $CoCl_2$, $MnCl_2$, $ZnCl_2$, $TiOSO_4$ and $Ce(NO_3)_4$. ATP was produced only when ferrous chloride was present in the solution. In the latter case, a white, colloidal material was precipitated after 5 h of reaction and the concentration of ADP decreased from 2×10^{-3} mol dm^{-3} to 0.97×10^{-3} mol dm^{-3}. The concentration of ATP formed was 0.23×10^{-3} mol dm^{-3} (ADP conversion = 11.5%). The difference between the amount of ADP consumed and that of ATP formed is believed to be incorporated in the precipitate, but details are left for further study. In subsequent experiments the amount of iron ion added was reduced to $\leqslant 0.5 \times 10^{-3}$ mol dm^{-3}, to avoid the precipitation.

Since the above reaction was carried out without deaeration, there was a possibility that the true active species was Fe^{III} ion, not Fe^{II}. In order to confirm this point, the effect of Fe^{II} and Fe^{III} ions was studied separately. Results obtained are shown in Table 1, which clearly indicates the active species in the phosphorylation reaction to be Fe^{III} and not Fe^{II}. In this case, an excess amount of hydroxylamine was added to the Fe^{II} solution to reduce any Fe^{III} ions which might be contained in the commercial reagent, and also to avoid aerial oxidation of Fe^{II}.

The course of the reaction over time was studied at three different temperatures, 23, 30 and 37 °C. In this case, the test solution (ADP, 1×10^{-3} mol dm^{-3}; AcP, 2×10^{-3} mol dm^{-3}; solvent, pure water; pH = 5.2) was first deaerated with nitrogen, and the reaction was started by addition of $Fe_2(SO_4)_3$ (0.24×10^{-3} mol dm^{-3}). Irrespective of the temperature, the solution pH was *ca.* 4.9 just after the addition of iron(II) salt but it decreased to, for instance, 4.7 after 4 h. During the reaction, the reactor vessel was vibrated to homogenize the solution. The results are shown graphically in Fig. 1. The reaction was complete within the first several hours, even at the lowest temperature, 23 °C. The curve for 37 °C declines after saturation. This is probably due to the hydrolysis of acetyl phosphate. The decrease in its concentration will shift the equilibrium to induce the dephosphorylation of ATP formed previously. Similar behaviour was observed by Saygin for the reaction of carbamyl phosphate with ADP.[2]

The percentage conversion of ADP was *ca.* 20%, which is comparable to the value reported by Saygin[5] for the calcium-ion-catalysed phosphorylation using carbamyl phosphate as the donor.

Fig. 2 shows the effect of Fe^{III} ion concentration. The two curves represent results obtained at two different concentrations of AcP. The most interesting feature of this figure is that the curves in the lower concentration region (less than *ca.* 0.15×10^{-3} mol dm^{-3}) lie above the stoichiometry line, which is indicated by a straight broken line. Since, in the duplicated measurements, the reproducibility was quite good and the error was within the size of the circular marks in Fig. 2, we may safely conclude that the Fe^{III} ion acts as a catalyst, not as a reagent. In addition, the peak point in the upper curve corresponds to a percentage conversion of *ca.* 25%, which is close to the highest value reported (28%)[4] for non-enzymatic ATP formation.

The conversion of ADP into ATP is thought to be caused by the high bonding energy of acetyl phosphate. Accordingly, we next studied the reaction using several high energy phosphates other than AcP. The phosphate donors studied were creatine, arginine and carbamyl phosphates. The use of inorganic orthophosphoric acid was also studied. Unfortunately, however, phosphorylation was observed only with creatine phosphate, and the corresponding percentage conversion of ADP was about 2%, while AcP converts 20% ADP. Similarly, Ca^{2+} ion, which had been reported to be an effective catalyst for the phosphorylation with carbamyl phosphate,[5] was found to be ineffective for the same reaction with AcP. In addition, it is worth noting that, even with AcP, phosphate transfer to AMP, to form ADP, was found to be impossible under the conditions suitable for ATP formation.

It is unclear how Fe^{III} ion catalyses the phosphorylation of ADP. Sayjin, who first reported that certain bivalent cations can catalyse phosphoryl transfer between carbamyl phosphate and ADP, suggested that the negative charge on the phosphate group is neutralized by the metal cation so as to open the possibility of nucleophilic attack of ADP on the phosphorus atom. This may be true, at least to some extent, but is clearly insufficient, since the phosphoryl transfer is also affected by the nature of the phosphate donor. Energetically, creatine phosphate [ΔG of hydrolysis (at $pH = 7$) $= -10.3$ kcal mol^{-1}] should be a better phosphorylating reagent than AcP ($\Delta G = -10.1$ kcal mol^{-1}).[6] Experimental results contradict this. In addition, Ca^{2+}, which was reported to catalyse the reaction of carbamyl phosphate, was found to be ineffective for the reaction of AcP, while the opposite result was obtained with Fe^{III}. For a full understanding of the mechanism more detailed studies are necessary.

Acknowledgements

We are grateful to Dr. Norio Takeuchi of National Chemical Lab., Tsukuba, for helpful discussions, and for supplying the ceramic electrode material.

References

1 N. Takeuchi, *J. Electrochem. Soc.*, 1989, **136**, 96.
2 O. Saygin, *Naturwiss.*, 1981, **68**, 617.
3 A. Todd and A. M. Michelson, Brit. Pat. 663 433, Dec. 19, 1951.
4 M. W. Hosseini and J-M. Lehn, *J. Chem. Soc., Chem. Commun.*, 1988, 397.
5 O. Saygin and E. Ellmauerer, *Origins of Life*, 1984, **14**, 139.
6 E. E. Conn and P. K. Stampf, *Outlines of Biochemistry*, 4th edn., Wiley, 1976, ch. 6.

Paper 0/02523B
Received 5*th June* 1990
Accepted 12*th October* 1990