PHOTOPHOSPHORYLATION OF RIBOSE MODIFIED ADP ANALOGS BY SPINACH CHLOROPLASTS

K. S. BOOS, B. DIMKE, E. SCHLIMME, H. WIEDNER[+], K. EDELMAN* and H. STROTMANN[†]

*Laboratorium für Biologische Chemie im Fachgebiet Organische Chemie der Universität (GH), 4790 Paderborn, [+]Boehringer Mannheim GmbH, Werk Penzberg, 8122 Penzberg, *Botanisches Institut, Abt. Biochemie der Pflanzen der Tierärtzlichen Hochschule, 3000 Hannover* and *[†]Lehrstuhl für Biochemische Pflanzenphysiologie der Universität, 4000 Düsseldorf, FRG*

Received 29 May 1981

1. Introduction

In [1–3] we reported on the properties of base-modified and phosphate chain-modified ADP analogs to serve as phosphoryl acceptors in photophosphorylation of isolated chloroplasts. Here, we extend the approach of mapping the ADP binding site of chloroplast ATP synthase by using substrate analogs to the action of ribose-modified nucleotides.

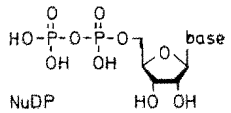
We know that the ADP molecule exhibits recognition centers in the heterocycle and in the diphosphate part [1–3] and that an intact pentofuranose ring is a prerequisite of the molecule in photophosphorylation [3]. Here, the effects of substituent modifications in C-2′ and C-3′ positions of the sugar moiety were investigated. We show that neither of the two hydroxyl groups is essential for ADP binding and phosphorylation. In accordance with [4–6], introduction of an electron-withdrawing group into C-3′ position yields ADP analogs which are powerful inhibitors of ADP phosphorylation. The results suggest that free rotation of the base around the *N*-glycosidic linkage as well as rotation around the C-4′–C-5′ bond and pseudo-rotation of the ribose ring are essential features of the nucleotide molecule with regard to recognition and catalysis by the active site of the enzyme.

2. Experimental

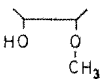
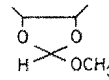
Chloroplasts from spinach leaves were prepared as in [7]. Photophosphorylation measurements were done as in [8]. The reaction medium contained 25 mM Tricine buffer (pH 8.0) 50 mM NaCl, 5 mM $MgCl_2$, 5 mM $^{32}P_i$, 50 μM phenazine methosulfate (PMS) and the nucleoside diphosphates at varying concentrations; chlorophyll was ~25 μg/ml. The illumination time was 5 s at a light intensity of 0.13 W/cm^2 (white light).

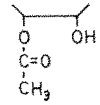
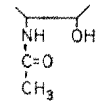
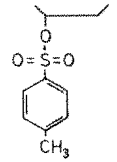
Chemical syntheses of the nucleoside 5′-diphosphates (table 1) were performed as follows: 2′,3′-methoxymethylidene ADP was prepared from ADP by reaction with trimethyl orthoformate according to [9]. rro ADP was synthesized as in [10]. The nucleoside diphosphates 3′-dADP and 3′-*O*-methyl-ADP were generated from the appropriate nucleosides in a one step reaction using $POCl_3$ in trimethylphosphates [11a] and 2′d,3′-tosyl- as well as 2′,3′-dideoxy-2′-,3′-didehydro-ADP were generated from the appropriate nucleosides in a one step reaction using $POCl_3$ in acetonitrile [11b]. The nucleoside diphosphates were isolated from the reaction mixture by DEAE-cellulose chromatography (3 × 50 cm) using a linear triethylammonium bicarbonate gradient (0–0.4 M, 3 l) 3′d,3′-amino adenosine was a gift of Dr Morr (GBF Braunschweig) to H. W. and was phosphorylated enzymatically to the appropriate 5′-diphosphate according to [12]. 3′d,3′-Azido-xyloadenosine was prepared as in [13], chemically phosphorylated to the corresponding 5′-diphosphate and subsequently reduced to the 3′d,3′-amino derivative by catalytic hydrogenation (Pd/Norit). 3′d,3′-*N*-acetyl-ADP was synthesized following the method used for the preparation of the bromoacetyl derivative of ADP [14]. The diphosphate moiety was identified by ^{31}P NMR spectroscopy (Bruker WP 60 DS, 60 MHz) and showed two groups of signals. The resonances centered at $\delta = -6$ (d,$J = 20$ Hz) for P2 and -11 (d,J = 20 Hz)

Table 1
Photophosphorylation of ribose-modified nucleoside-5′-diphosphates



Base: adenine (A); guanine (G)

NuDP			Rel. K_m (μM)	Rel. V_{max}
ADP	1		1.0	1.00
3′d-ADP	2		1.0	1.00
2′d-ADP	3		1.0	1.00
2′,3′-dd-ADP	4		1.0	1.00
2′,3′dd-2′,3′-didehydro-ADP	5		(a) Not phosphorylated (b) Not an inhibitor	
ara-ADP	6		3.0	0.63
rro-ADP	7		(a) Not phosphorylated (b) Very poor inhibitor	
3′-*O*-methyl-ADP	8		1.0	1.00
2′-*O*-methyl-ADP	9		1.0	1.00
2′,3′-methoxy-methylidene-ADP	10		(a) Not phosphorylated (b) Not an inhibitor	

NuDP			Rel. K_m (μM)	Rel. V_{max}
3′-*O*-acetyl-ADP	11		(a) Not phosphorylated [5] (b) I_{50} = 85 μM (100 μM ADP)	
3′d,3′-amino-ADP	12		1.0	0.69
3′d-3′-aminoxylo-ADP	13		10.5	0.28
3′d,3′-*N*-acetyl-ADP	14		(a) Not phosphorylated (b) K_i = 10 μM	
2′d,3′-tosyl-ADP	15		(a) Not phosphorylated (b) K_i = 45 μM	
GDP	16		3.9	1.00
2′d-GDP	17		7.3	1.00

K_i-values were calculated from the double reciprocal plots according to: $K_i = [I] \cdot K_m/(K_{m'} - K_m)$

K_m- and V_{max}-values for ADP were in the range of: K_m = 41–70 μM

V_{max} = 500–1080 μmol $^{32}P_i$ incorporated . mg chl^{-1} . h^{-1}

ppm for P1 upfield to 85% H_3PO_4 as external standard. 2′d,3′-tosyl- as well as 2′,3′-dideoxy-2′,3′-didehydro adenosine were prepared according to [15]. All other nucleoside diphosphates and enzymes used were commercially available from Boehringer (Mannheim). 2′,3′-Dideoxy-ADP and 2′-*O*-methyl ADP were prepared from the corresponding 5′-triphosphates (P-L Biochemicals, Milwaukee WI) by treatment with hexokinase (EC 2.7.1.1) as in [3]. 3′-*O*-Methyladenosine was a gift from Dr J. W. Daly (NIH, Bethesda MD).

3. Results and discussion

The kinetic parameters K_m and V_{max} for the employed ADP analogs were determined under comparable experimental conditions using PMS as a mediator of a cyclic electron-transport system. Nevertheless, K_m and V_{max} varied considerably from experiment to experiment, in particular if chloroplasts from winter and summer spinach were compared. Therefore in every experiment a concentration curve with ADP was run as a control and the relative V_{max} and K_m values, respectively, were computed according to [16]:

$$\text{rel. } V_{max\,(NuDP)} = V_{max\,(NuDP)}/V_{max\,(ADP)}$$

$$\text{rel. } K_m = K_{m\,(NuDP)} \cdot V_{max\,(ADP)}/K_{m\,(ADP)} \cdot V_{max\,(NuDP)}$$

The relative K_m values can be taken as approximate measures for the affinities of the substrate analogs to the active site of ATP synthase [2,16]. If an analog was found to be not a substitute for ADP in phosphorylation, its competitive effect on ATP synthesis was tested. The app. K_i values are noted in table 1.

According to the obtained results shown in table 1, the ribose-modified ADP analogs may be subdivided into 3 classes:

(1) Compounds which act as substrates instead of ADP (1–4,6,8,9,12,13);
(2) Compounds which are not substrates but competitive inhibitors of ADP phosphorylation (11,14,15);
(3) Compounds which are neither substrates nor inhibitors (5,7,10).

Substitution of the 2′- or 3′-hydroxyl-groups or both by hydrogen neither affects the app. K_m nor the V_{max} value. Methylation of either of the groups is likewise without any effect on these two parameters (compounds 2–4,8,9). Replacement of the 3′-hydroxyl by an amino group (compound 12) reduces V_{max} but not the relative K_m. These results indicate that the OH-groups in position 2′ and 3′ are meaningless for recognition and reactivity of the ADP molecule on the active site of chloroplast ATP synthase. However, inversion of the substituents on C-2′ as in arabino-ADP, leads to an increase in relative K_m as well as a decrease in V_{max}. A similar but still more pronounced change is observed with the stereoisomer of the 3′-amino-substituted ADP analog (compound 13). Substituent inversion in the indicated positions greatly restricts free rotations around the C-4′–C-5′ bond and the *N*-glycosidic linkage [17,18]. It may be concluded that an unrestricted orientation of these parts of the nucleotide molecule is a prerequisite for accomodation in the protein counterpart. Reduction in V_{max} means that the catalytic process including product release is inhibited. Since V_{max} is decreased upon substituent inversion at C-2′ or C-3′, we may suggest that the formation of ATP on the enzyme or its release likewise require free rotation of the base and phosphate moiety, respectively.

The group of inhibitory analogs includes C-3′-acyl- and arylesters of ADP [5,19–22]. They act as energy transfer inhibitors in photophosphorylation as well as in oxidative phosphorylation [4–6]. The mode of inhibition is competitive with regard to ADP, suggesting that they occupy the active ADP binding sites of the corresponding ATP synthases. In particular those analogs which bear an unpolar substituent, were found to inhibit ADP phosphorylation in a rather low concentration range, suggesting that a hydrophobic interaction might additionally increase the stability of the enzyme–analog complex.

Compound 15 is a 2′-deoxyribonucleotide analog, i.e., a 2′ ⇌ 3′ acyl migration as known from ribonucleotide derivatives [23] is excluded. These results thus indicate that any ADP analog which contains a strong-electron withdrawing substituent in the C-3′ region of the ribose moiety as for example a carbonyl-, amide- or sulfonyl-group is potential inhibitor of phosphorylation. Hence the negative inductive effect may be the reason why these molecules are unable to act as phosphoryl acceptors.

The third group of compounds is represented by

two analogs (5,10) which both contain a rather inflexible pentofuranose ring, thus preventing or altering pseudo-rotation, i.e., 2',3'-endo ⇌ exo transitions [18,24]. This is attained by introduction of a 2',3'-double bond (5), or by cyclisation of the vicinal hydroxyls (10), respectively. Although pseudo-rotation does not contribute too much to the flexibility of the whole nucleotide molecule, both chemical modifications distort, at least, the overall molecular geometry compared to ADP. This distortion does not allow an adjustment of a specific positioning of the adenine moiety and the phosphate chain relative to each other, which is a prerequisite for the active site of the ATP synthase to contact base and phosphate chain in a correct manner.

The ribose ring-opened ADP is likewise an almost inactive analog. This molecule assumes a conformation which is quite different from a normal nucleotide structure due to the presence of additional rotational axes in the sugar moiety [25].

These results indicate that the ribose moiety of the ADP molecule is not involved in binding of the nucleotide to the active site of chloroplast ATP synthase. However, its structural role is to facilitate the adoption of a specific substrate conformation which permits attachment to the protein counterpart with its recognition sites heterocycle and diphosphate moiety.

Acknowledgements

This work was supported by grants of the Deutsche Forschungsgemeinschaft and the Fonds des Verbandes der Chemischen Industrie.

References

[1] Strotmann, H., Bickel-Sandkötter, S., Edelmann, K., Eckstein, F., Schlimme, E., Boos, K. S. and Lüstorff, J. (1979) Biochim. Biophys. Acta 545, 122.
[2] Schlimme, E., de Groot, E. J., Schott, E., Edelmann, K. and Strotmann, H. (1976) FEBS Lett. 106, 251.
[3] Boos, K. S., Lüstorff, J., Schlimme, E., Hesse, H. and Strotmann, H. (1976) FEBS Lett. 71, 124.
[4] Schäfer, G., Onur, G., Edelmann, K., Bickel-Sandkötter, S. and Strotmann, H. (1978) FEBS Lett. 87, 318.
[5] Schäfer, G., Onur, G. and Strotmann, H. (1978) in: Energy Conservation in Biological Membranes (Schäfer, G. and Klingenberg, M. eds) p. 220, Springer-Verlag Heidelberg.
[6] Schäfer, G. and Onur, G. (1979) Eur. J. Biochem. 97, 415.
[7] Strotmann, H. and Bickel-Sandkötter, S. (1977) Biochim. Biophys. Acta 460, 126.
[8] Strotmann, H. (1970) Ber. Deutsch. Bot. Ges. 83, 443.
[9] Chládek, S. (1968) in: Synthetic Procedures in Nucleic Acid Chemistry (Zorbach, W. W. and Tipson, R. S. eds) vol. 1, p. 292, Wiley, New York.
[10] Boos, K. S., Schlimme, E., Bojanovski, D. and Lamprecht, W. (1975) Eur. J. Biochem. 60, 451.
[11a] Yoshikawa, M., Kato, T. and Takenishi, T. (1967) Tetrahedron Lett. 50, 5065.
[11b] Sowa, T. and Ouchi (1975) Bull. Chem. Soc. Japan 48, 2084.
[12] Fraser, Th. H. and Rich, A. (1973) Proc. Natl. Acad. Sci. USA 70, 2671.
[13] Robins, M. J., Fouron, Y. and Mengel, R. (1974) J. Org. Chem. 39, 1564.
[14] Armstrong, V. W. and Eckstein, F. (1979) Biochemistry 18, 5117.
[15] McCarthy, J. R., Robins, M. J., Townsend, L. B. and Robins, R. K. (1966) J. Am. Chem. Soc. 88, 1549.
[16] Franek, U. and Strotmann, H. (1981) FEBS Lett. 126, 5.
[17] Remin, M., Darzynkiewicz, E., Dworak, A. and Shugar, D. (1976) J. Am. Chem. Soc. 98, 367.
[18] Davies, D. B. (1978) Prog. Nucl. Magn. Reson. Spect. 12, 135.
[19] Russel, J., Jeng. S. J. and Guillory, R. J. (1976) Biochem. Biophys. Res. Commun. 70, 1225.
[20] Lunardi, J., Lauquin, G. J. M. and Vignais, P. (1977) FEBS Lett. 80, 317.
[21] Scheurich, P., Schäfer, H. J. and Dose, K. (1978) Eur. J. Biochem. 88, 253.
[22] Schäfer, H. J., Scheurich, P., Ratgeber, G. and Dose, K. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 324.
[23] Reese, C. B. and Trentham, D. R. (1965) Tetrahedron Lett. 23, 2467.
[24] Hutcheon, W. L. B. and James, M. N. G. (1974) Acta Cryst. B30, 1777.
[25] Van der Haar, F., Schlimme, E., Gomez-Guillen, M. and Cramer, F. (1971) Eur. J. Biochem. 24, 296.