Volume 231, number 1, 259-262 FEB 05745 April 1988

Anthraquinone inhibitors of photosystem II electron transport

Walter Oettmeier, Klaus Masson and Andreas Donner

Lehrstuhl Biochemie der Pflanzen, Fakultät für Biologie, Ruhr-Universität, Postfach 10 21 48, D-4630 Bochum 1, FRG

Received 10 February 1988

Various substituted 9,10-anthraquinones were tested for their inhibitory activity on photosystem II electron transport. Maximal inhibitory activity was achieved if the positions adjacent to one of the quinone carbonyl groups were unsubstituted or substituted by hydroxyl groups only. The best anthraquinone type inhibitor found so far was 2,3,4-trichloro-1-hydroxy-anthraquinone with a pI_{50} value of 7.75. This is well comparable to the most powerful known photosystem II inhibitors. As studied by binding experiments with 1-[^{14}C]methoxy-anthraquinone, after covalent modification of thylakoids with azido-atrazine, anthraquinones bind at the photosystem II D1 protein. Their orientation within the binding niche seems to be different from that of other photosystem II inhibitors.

9,10-Anthraquinone; Photosystem II; Binding characteristic; Covalent modification

1. INTRODUCTION

In photosystem II electron transport the primary (Q_A) and secondary (Q_B) electron acceptors are plastoquinone molecules. The 'two-electron gate' intermediate Q_B connects the one-electron carrier Q_A with the two-electron carrier plastoquinone pool [1,2]. Photosystem II inhibitors, some of which are used as efficient herbicides, compete with plastoquinone for binding at the secondary quinone acceptor site (Q_B site) to the D1-protein [3]. Photosystem II inhibitors in general are not quinoid compounds but belong to different chemical classes. However, due to their close relationship with the native plastoquinone quinoid compounds as inhibitors of photosynthetic electron transport are of special interest. We have recently reported on the inhibitory properties of 1,4-benzoquinones and -naphthoquinones on the Q_B site [4–7]. As judged from QSAR [4,5] their binding site, i.e., their orientation within the binding niche, seems to be different from that of other photosystem II inhibitors.

Anthraquinones have been extensively studied in reaction centres from photosynthetic bacteria, where they can substitute for the native Q_A [8,9]. Furthermore, anthraquinones were shown to be efficient quenchers of chlorophyll fluorescence in isolated chloroplasts [10]. We wish to report here that certain substituted 9,10-anthraquinones are potent inhibitors of photosystem II electron transport. In their inhibitory activity they are comparable to the most powerful photosystem II herbicides. Furthermore, in anthraquinones a total of 8 positions is available for substitution. This allows for a great variety of structures and, therefore, anthraquinones are an especially suitable tool for elucidation of the inhibitor binding niche. Finally, a 1-[^{14}C]methoxy-9,10-anthraquinone has been synthesized and its binding properties to the thylakoid membrane have been examined after covalent modification with azido-atrazine.

Correspondence address: W. Oettmeier, Lehrstuhl Biochemie der Pflanzen, Postfach 10 21 48, D-4630 Bochum 1, FRG

Abbreviations: chl, chlorophyll; DCIP, dichlorophenolindophenol; DNP-INT, 2-iodo-2′,4,4′-trinitro-3-methyl-6-isopropyldiphenyl ether; QSAR, quantitative structure-activity relationship

Published by Elsevier Science Publishers B.V. (Biomedical Division)

2. MATERIALS AND METHODS

So far available, anthraquinones have been purchased from Aldrich Chemie (including chemicals from the Alfred Bader Chemical Division). Dinitro-anthraquinones were generous gifts from Dr Stawitz, Bayer AG, Leverkusen. All other anthraquinones were synthesized according to standard procedures [11]. 1-[^{14}C]Methoxy-9,10-anthraquinone was synthesized by methylation of 1-hydroxy-anthraquinone with [^{14}C]methyliodide under phase transfer catalysis conditions.

Chloroplasts from spinach were prepared according to [12] and stored in liquid nitrogen in the presence of 10% glycerol. pI_{50} values were determined in the system H_2O > DCIP [13] in the presence of 1 μM DNP-INT [14] in order to prevent electron flow through photosystem I. The binding experiments with 1-[^{14}C]methoxy-anthraquinone were performed essentially as described [7]. The protocol for binding experiments after covalent modification of the photosystem II inhibitor binding site is described in [15].

3. RESULTS AND DISCUSSION

Table 1 lists pI_{50} values for various 9,10-anthraquinones in the system H_2O > DCIP in the presence of DNP-INT [14]. DNP-INT blocks electron flow at the cytochrome b_6/f complex and DCIP reduction by photosystem I is prevented. Table 1 shows that unsubstituted anthraquinone (no.1) is no inhibitor at all. Introduction of a substituent in the 1-position leads to inhibitory active compounds, the methoxy- and iodo-substituents (nos 9,10) being the most effective ones. As is true for other photosystem II inhibitors, activity increases from the chloro- (no.7) to the iodo-substituent (no.10). Similarly, introduction of a substituent in the 2-position also yields active substances. However, from the compounds tested so far, the 2-substituted anthraquinones are less active as compared to the 1-substituted ones.

If a second substituent is added to the quinone moiety, inhibitory activity generally increases, provided certain positions are left free or are substituted by particular substituents. This is demonstrated in the case of the dinitro-anthraquinones (table 1c). 1,5-Dinitro-anthraquinone is completely inactive, the 1,8-isomer is moderately active and both 1,6- and 1,7-isomers are highly active. Furthermore, if one compares the biological activities of 1-methoxy- and 1,4-dimethoxy-anthraquinone (nos 9 and 23, respectively) it is evident that the introduction of a second methoxy group in the 4-position renders the compound completely inactive. These results indicate that at least for one quinone carbonyl position the adjacent positions, i.e. the 4- and 5-position, should be left unsubstituted in order to achieve high inhibitory activity. However, there is one exception to this general rule. Compounds 24–37 in table 1d are all highly active. They have in common that they contain at least one hydroxyl group. As is seen from the substitution pattern of highly active com-

Table 1

pI_{50} values for inhibition of photosystem II electron transport by various 9,10-anthraquinones

No.	-9,10-anthraquinone	pI_{50} value
a. 1-substituted		
1		<2.00
2	1-nitro-	3.68
3	1-amino-	4.49
4	1-acetoxy-	4.53
5	1-hydroxy-	5.01
6	1-ethoxy-	5.09
7	1-chloro-	5.21
8	1-azido-	5.48
9	1-methoxy-	6.37
10	1-iodo-	6.59
b. 2-substituted		
11	2-carboxy-	4.24
12	2-methyl-	4.28
13	2-hydroxymethyl-	4.50
14	2-carboxymethyl-	4.96
15	2-chloromethyl-	5.18
16	2-azido-	5.44
17	2-ethyl-	5.53
18	2-tert.butyl-	5.72
c. Dinitro-anthraquinones		
19	1,5-dinitro-	<2.00
20	1,8-dinitro-	5.36
21	1,6-dinitro-	6.84
22	1,7-dinitro-	6.89
d. Hydroxy- and methoxy-anthraquinones		
23	1,4-dimethoxy-	<2.00
24	1-hydroxy-4-methoxy-	5.38
25	1,2-dibromo-4-hydroxy-	5.64
26	1,8-dihydroxy-	5.70
27	2,4-dichloro-1-hydroxy-	5.74
28	1,4-dihydroxy-	5.88
29	1-hydroxy-8-methoxy-	5.90
30	1,2-dihydroxy-	6.21
31	1,2-dihydroxy-3-nitro-	6.24
32	1,2,4-trihydroxy-	6.31
33	1,4-dihydroxy-2-acetoxy-	6.34
34	3-bromo-1,2-dihydroxy-	6.82
35	1-bromo-4-hydroxy-	6.86
36	1,3-dichloro-2-hydroxy-	6.89
37	2,3,4-trichloro-1-hydroxy-	7.75

pounds 24–37, a hydroxyl group in a position adjacent to one of the quinone carbonyl groups does not result in a loss of activity but even increases activity. Obviously, a hydroxyl group is favourable in close neighbourship to the carbonyl group either due to its small van der Waals radius or due to specific hydrogen bonding to a suitable amino acid within the binding niche. Our best anthraquinone inhibitor, 2,3,4-trichloro-1-hydroxy-anthraquinone with a pI_{50} value of 7.75 in its inhibitory potency is equivalent to the cyanoacrylate type inhibitors, the best photosystem II inhibitors known so far [16].

Preliminary QSAR calculations suggest that steric and electronic parameters and not the lipophilicity govern the biological activity. This is the same situation as for benzo- and naphthoquinones [4,5].

In order to study the binding properties of anthraquinones, a 1-[^{14}C]methoxy-anthraquinone has been synthesized. Its binding behaviour exhibits the same characteristics, specific and unspecific binding, like other photosystem II inhibitors [17,18]. A binding constant K_b = 0.14 (± 0.02) μM (corresponding to a pK_b of 6.87 (± 0.06) has been determined, which corresponds well to the pI_{50} value of 6.4 (the latter is not extrapolated to zero chlorophyll concentration). The number of binding sites x_t was calculated 3.32 (± 0.36) nmol/mg chlorophyll, which is equivalent to 1 molecule of inhibitor per 339 (± 37) molecules of chlorophyll or about one molecule of inhibitor per electron transport chain.

In another type of binding experiment, the photosystem II inhibitor binding site was modified by covalent linkage of the nitrene generated from azido-atrazine by UV-illumination [15]. Subsequently, 1-[^{14}C]methoxy-anthraquinone binding was studied (fig.1). As can be seen from fig.1A, upon increasing amounts of prebound azido-atrazine the fraction of bound 1-[^{14}C]methoxy-anthraquinone diminishes. The specific binding is much more affected than the unspecific binding. The Lineweaver-Burk plot of the binding data (fig.1B) reveals no change in the number of binding sites x_t (identical ordinate intercepts), but a change in the binding constants K_b (different abscissa intercepts). A similar result was obtained by Vermaas et al. [19] for atrazine and ioxynil binding after covalent modification by an azido-

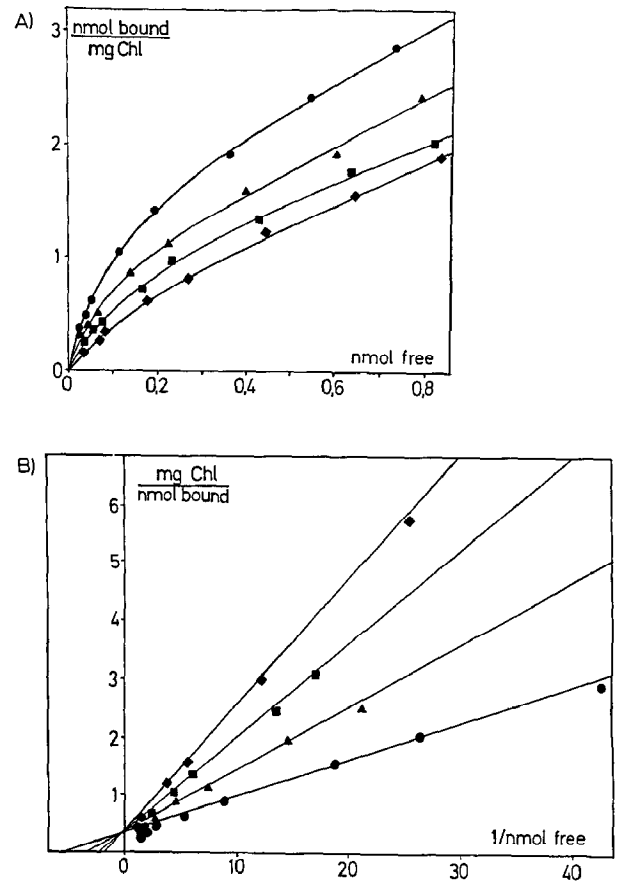

Fig.1. Binding of 1-[^{14}C]methoxy-anthraquinone to thylakoids after covalent modification with azido-atrazine. (●—●) Control; (▲—▲) + 1 nmol; (■—■) + 2 nmol; (◆—◆) + 3 nmol/mg chl. (A) Binding curves. (B) Lineweaver-Burk plot.

benzoquinone. These results further corroborate the notion as obtained by QSAR analysis that the binding sites for quinones in general are different – though possibly overlapping – from those of other photosystem II inhibitors.

Acknowledgements: This work was supported by Deutsche Forschungsgemeinschaft. We are indebted to Dr Stawitz, Bayer AG, Leverkusen, for the generous gift of dinitro-anthraquinones.

REFERENCES

[1] Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 314, 250–256.
[2] Velthuys, B.R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94.

[3] Velthuys, B.R. (1981) FEBS Lett. 126, 277–281.
[4] Soll, H.J. and Oettmeier, W. (1984) in: Advances in Photosynthesis Research (Sybesma, C. ed.) vol.4, pp.5–8, Martinus Nijhoff/Dr W. Junk Publishers, The Hague, Boston, Lancaster.
[5] Oettmeier, W., Dierig, C. and Masson, K. (1986) Quant. Struct.-Act. Relat. 5, 50–54.
[6] Oettmeier, W., Masson, K. and Dostatni, R. (1987) Biochim. Biophys. Acta 890, 260–269.
[7] Oettmeier, W., Dostatni, R. and Santel, H.J. (1987) Z. Naturforsch. 42c, 693–697.
[8] Gunner, M.R., Robertson, D.E. and Dutton, P.L. (1986) J. Phys. Chem. 90, 3783–3795.
[9] Gunner, M.R., Robertson, D.E., LoBrutto, R.L., McLaughlin, A.C. and Dutton, P.L. (1987) in: Progress in Photosynthesis Research (Biggins, J. ed.) vol.I, pp.217–220, Martinus Nijhoff Publishers, Dordrecht.
[10] Karukstis, K.K., Gruber, S.M., Fruetel, J.A. and Boegemann, S.C. (1988) Biochim. Biophys. Acta 932, 84–90.
[11] Houben-Weyl, Methoden der Organischen Chemie (1979) Band VII/3c, Georg Thieme Verlag, Stuttgart.
[12] Nelson, N., Drechsler, Z. and Neumann, J. (1970) J. Biol. Chem. 245, 143–151.
[13] Oettmeier, W., Godde, D., Kunze, B. and Höfle, G. (1985) Biochim. Biophys. Acta 807, 216–219.
[14] Trebst, A., Wietoska, H., Draber, W. and Knops, H.J. (1978) Z. Naturforsch. 33c, 919–927.
[15] Oettmeier, W., Kude, C. and Soll, H.J. (1987) Pestic. Biochem. Physiol. 27, 50–60.
[16] Huppatz, J.L. and Phillips, J.N. (1987) Z. Naturforsch. 42c, 679–683.
[17] Tischer, W. and Strotmann, H. (1977) Biochim. Biophys. Acta 460, 113–125.
[18] Laasch, H., Pfister, K. and Urbach, W. (1982) Z. Naturforsch. 37c, 620–631.
[19] Vermaas, W.F.J., Arntzen, C.J., Gu, L.Q. and Yu, C.A. (1983) Biochim. Biophys. Acta 723, 266–275.