Biochimica et Biophysica Acta, 682 (1982) 496–499
Elsevier Biomedical Press

BBA Report

BBA 40015

CLASSIFICATION OF PHOTOSYSTEM II INHIBITORS BY THERMODYNAMIC CHARACTERIZATION OF THE THERMOLUMINESCENCE OF INHIBITOR-TREATED CHLOROPLASTS

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(Received June 29th, 1982)

Key words: Electron transfer; Photosystem II inhibitor; Thermoluminescence; Activation energy; Activation entropy; Photosynthesis

Computer-assisted curve resolution was used to analyse the thermoluminescence of chloroplasts treated by different inhibitors in order to determine the free energy of activation, ΔF, activation energy, ΔE, and activation entropy, ΔS, of the radiative charge-recombination reaction. It was found that the activation energies and entropies related to the different inhibitor treatments of chloroplasts exhibit a compensation relationship. On the basis of the values of the activation parameters ΔE and ΔS, the Photosystem II inhibitors, which block electron transport between the primary acceptor Q and plastoquinone, could be classified into two main groups.

A large number of photosynthetic inhibitors block the electron flow in PS II between the primary acceptor, Q, and the plastoquinone pool (in the following they will be called PS II inhibitors) [1]. In the presence of PS II inhibitors the thermoluminescence of isolated chloroplasts originates in the radiative charge recombination of the positively charged S_3 state of the water-splitting enzyme and the negatively charged primary acceptor, Q^- [2]. The activation energy, ΔE, and activation entropy, ΔS, of the charge-recombination process can be determined by mathematical analysis of the glow curve [3]. Inhibitor binding to the chloroplast membrane has a strong influence on the thermoluminescence and in consequence on the activation parameters (ΔE and ΔS).

The purpose of the present work was to classify the PS II inhibitors (the majority of which are also used as photosynthetic herbicides) on the basis of the values of activation parameters obtained by computer analysis of the thermoluminescence of inhibitor-treated chloroplasts.

Chloroplasts were isolated from maize as described earlier [4]. Samples for thermoluminescence measurements contained 55% glycerol to prevent the distortion of the glow curves by the solid-liquid phase transition of water. Computer analysis of glow curves was carried out as described in Ref. 3.

According to their chemical structures the PS II inhibitors can be classified into two groups. The first group is comprised of a variety of chemically different compounds which have a common structural element, the $-\overset{|}{C}-\overline{N}-$ group (DCMU-type inhibitors) [1]. Recently, a new group of inhibitors was discovered, the substituted nitrophenols and other phenols (phenolic inhibitors). These inhibitors do not have an essential element analogous to the inhibitory group of DCMU-type inhibitors [1].

The effect of DCMU-type and phenolic inhibitors on thermoluminescence of isolated chloroplasts was investigated. Using a computer-assisted

Abbreviations: PS, photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

0005-2728/82/0000–0000/$02.75

TABLE I

THERMODYNAMIC CHARACTERISTICS OF THE THERMOLUMINESCENCE BANDS OF INHIBITOR-TREATED CHLOROPLASTS

I_{max}, temperature at the maximum of the thermoluminescence band, ΔE, activation energy, ΔS, activation entropy, ΔF, free energy of activation. $\bar{X}$ represents the mean value and the standard deviations are shown in brackets. In the table the trivial names of the herbicides are given.

Herbicide	T_{max} (K)	ΔE (eV)	ΔS ($\times 10^4$) (eV/K)	ΔF (25°C) (eV)
DCMU-type inhibitors				
Ureas				
Diuron (DCMU)	280	0.731	−2.06	0.792
Monuron	278	0.707	−3.29	0.805
Linuron	280	0.702	−3.09	0.794
Chlortoluron	279	0.713	−2.57	0.789
Chloroxuron	281	0.714	−2.83	0.798
Chlorbromuron	278	0.736	−1.67	0.784
Triazines				
Atrazine	277	0.670	−4.01	0.789
Simazine	274	0.661	−4.70	0.801
Cyanazine	276	0.694	−3.08	0.786
Aziprotryn	281	0.671	−4.06	0.792
Terbutryn	278	0.687	−3.53	0.792
Metribuzin	279	0.728	−2.12	0.791
Phenylcarbamates				
Phenmedipham	279	0.724	−2.75	0.806
Pyridazinones				
Pyrazon	277	0.707	−3.27	0.806
Uracils				
Lenacil	276	0.670	−3.97	0.789
$\bar{X}$	278.2	0.701	−3.13	0.794
	(±2.0)	(±0.025)	(±0.84)	(±0.007)
Phenolic inhibitors				
Hydroxybenzonitriles				
Bromoxynil	264	0.595	−6.00	0.773
Ioxynil	265	0.625	−4.97	0.773
Dinitrophenols				
2,4-Dinitrophenol	267	0.592	−5.89	0.775
Dinoseb	266	0.603	−6.13	0.786
DNOC	266	0.598	−6.24	0.784
Trinitrophenols				
Picric acid	267	0.648	−4.26	0.775
$\bar{X}$	265.8	0.610	−5.58	0.778
	(±1.2)	(±0.022)	(±0.79)	(±0.006)

curve-fitting technique the activation energies, activation entropies and free energies of activation related to the thermoluminescence bands were determined. The results are listed in Table I.

The activation entropies are negative, indicating that in line with the proposal of Mar and Roy [5] in the charge-separation process a part of the light energy absorbed is stored reversibly as an increase in the entropy of the environment surrounding the separated charges. Conversely, the charge recombi-

nation resulting in thermoluminescence emission involves a decrease in entropy due to the rearrangement of the loosened membrane structure back to its original state. A similar decrease in entropy was obtained in the charge-recombination reaction resulting in delayed fluorescence emission of *Rhodopseudomonas sphaeroides* and *Rps. viridis* [6–8].

Comparison of the activation energies and activation entropies related to the different inhibitors shows that they vary in a parallel fashion, i.e., a decrease in activation energy leads to a proportional lowering in the activation entropy. This compensation effect resulted in relatively little changes in the free energy of activation. The free energy of activation is determined by the redox span between the interacting donor and acceptor molecules participating in the radiative charge recombination [3]. With all inhibitor-treated chloroplasts the S_3 state is involved in the generation of thermoluminescence bands [2], therefore the small differences in the free energies of activation suggest that only one acceptor (Q) participates in the charge recombination and, moreover, that its midpoint potential is slightly different in the presence of DCMU-type and phenolic inhibitors.

A plot of activation entropies against activation energies shows more clearly than the data in Table I that so called 'compensation' or 'isokinetic' correlation [9] exists among the activation parameters of radiative charge-recombination reactions occurring in inhibitor-treated chloroplasts (Fig. 1).

The relationship between ΔE and ΔS can be characterized by the compensation or isokinetic temperature (T_c) derived from the least-squares slope of this plot [9]. Curve fitting of the data points gives two straight lines; one for the DCMU-type and one for the phenolic inhibitors (Fig. 1). It can be seen that on the basis of the activation parameters (ΔE and ΔS), the inhibitors can be classified into the same two groups which were distinguished according to similarities in their chemical structures. The DCMU-type inhibitors belong to the first group, the phenolic inhibitors to the second.

In our earlier work, neglecting entropy changes, the ureas and triazines were classified into different groups and thus three herbicide classes were

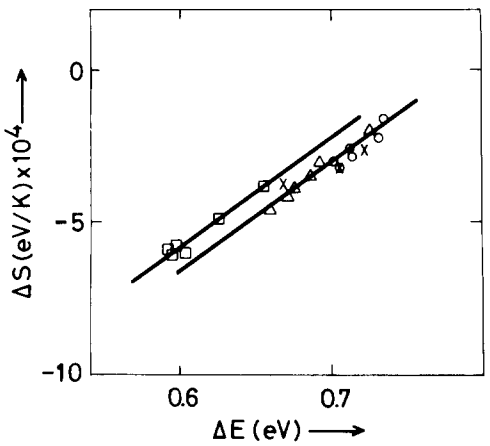

Fig. 1. Isokinetic correlation for the radiative charge-recombination reaction (thermoluminescence) in inhibitor-treated chloroplasts. (○) Ureas, (△) triazines, (×) other DCMU-type inhibitors, (□) phenolic inhibitors. Data are taken from Table I. The isokinetic temperatures are $T_c \approx 304$ K for the DCMU-type and $T_c \approx 283$ K for the phenolic inhibitors.

distinguished [10]. However, as Fig. 1 shows, considering both the activation energies and entropies, the ureas (○) and triazines (△) lie on the same compensation line suggesting that they should belong to one herbicide class.

The classification of PS II inhibitors into two groups according to the values of activation parameters is supported by recent theoretical studies of Trebst et al. [11]. They have found by molecular orbital calculations that the DCMU-type and phenolic inhibitors differ significantly in the charge distribution at the atoms essential for binding to the chloroplast membrane.

The action mechanism of the PS II herbicides has not yet been clarified. According to the present view, the PS II herbicides do not react directly with an electron carrier of the electron-flow system but bind noncovalently to a protein in which the Q and B electron-transport components are embedded [1].

We suggest that the interactions of the different inhibitors (belonging to the same herbicide class, but representing different chemical structures) with the herbicide-binding protein induce various alterations in the state of Q which in consequence are reflected as a compensation relationship in the activation parameters of the charge-recombination reaction.

The existence of two compensation relationships among the activation parameters for the two different inhibitor classes indicates that the DCMU-type and phenolic inhibitors bind to two different domains of the herbicide-binding protein. Interactions of inhibitors with the two domains of the protein represent two reaction series with related reactions within one series, thus displaying two compensation relationships. This conclusion is consistent with recent evidence according to which the binding and inhibition sites of DCMU-type and phenolic herbicides are located on two different polypeptides of the herbicide-binding protein [12,13].

This work was supported by Contract No. 366/82/1.6 by the Central Research Fund of the Hungarian Academy of Sciences. The authors wish to thank Dr. Grinius Vytautas for his kind gift of several herbicides.

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