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Two-electron reduction of nitroaromatic compounds by *Enterobacter cloacae* NAD(P)H nitroreductase: Description of quantitative structure–activity relationships★✪

Henrikas Nivinskas[1], Ronald L. Koder[2], ilvinas Anusevičius[1], Jonas Šarlauskas[1], Anne-Frances Miller[2] and Narimantas Čenas[1]✉

[1]*Institute of Biochemistry, Mokslininku 12, Vilnius 2600, Lithuania;* [2]*Department of Chemistry, University of Kentucky, 106 Chemistry-Physics Building, Lexington, KY 40506-0055, U.S.A.*

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***Enterobacter cloacae* NAD(P)H : nitroreductase catalyzes the reduction of a series of nitroaromatic compounds with steady-state bimolecular rate constants (k_{cat}/K_m) ranging from 10^4 $M^{-1}s^{-1}$ to 10^7 $M^{-1}s^{-1}$, and oxidizing 2 moles NADH per mole mononitrocompound. Oxidation of excess NADH by polynitrobenzenes including explosives 2,4,6-trinitrotoluene (TNT) and 2,4,6-trinitrophenyl-*N*-methylnitramine (tetryl), has been observed as a slower secondary process, accompanied by O_2 consumption. This type of 'redox cycling' was not related to reactions of nitroaromatic anion-radicals, but was caused by the autoxidation of relatively stable reaction products. The logs k_{cat}/K_m of all the compounds examined exhibited parabolic dependence on their enthalpies of single-electron- or two-electron (hydride) reduction, obtained by quantum mechanical calculations. This type of quantitative structure–activity relationships shows that the reactivity of nitroaromatics towards *E. cloacae* nitroreductase depends mainly on their hydride accepting properties, but not on their particular structure, and does not exclude the possibility of multistep hydride transfer.**

Nitroaromatic compounds are widely used as pharmaceuticals, pesticides, explosives, and comprise an important group of environmental pollutants [1]. For the manifestation

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✉Corresponding author: phone (37 02) 729 042, fax: (37 02) 729 196, e-mail: ncenas@bchi.lt

Abbreviations: DT-diaphorase, (NAD(P)H:quinone reductase; ΔHf, enthalpy of reaction; E^1_7, single-electron reduction potential at pH 7.0; HMX, octohydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; k_{cat}, catalytic constant; k_{cat}/K_m, bimolecular rate constant; NR, *Enterobacter cloacae* nitroreductase; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine; TNT, 2,4,6-trinitrotoluene; TNC, 1,3,6,8-tetranitrocarbazole.

of their therapeutic and/or cytotoxic properties, most nitroaromatics should undergo single- or two-electron enzymatic reduction in the organism. Single-electron reduction of nitroaromatics is catalyzed by flavoenzymes dehydrogenases-electrontransferases, e.g., NADPH : cytochrome P-450 reductase (EC 1.6.2.4), ferredoxin : $NADP^+$ reductase (EC 1.18.1.2), NADH : ubiquinone reductase (EC 1.6.99.3), bacterial oxygen-sensitive nitroreductases [2–6]. Under aerobiosis, single-electron reduction of nitroaromatics to their anion-radicals results in their reoxidation by oxygen with the formation of superoxide and, subsequently, hydrogen peroxide and hydroxyl radical, that damage proteins, nucleic acids and lipids. Two-electron reduction of nitroaromatics to nitroso compounds and, subsequently, to hydroxylamines, is catalyzed by mammalian DT-diaphorase (NAD(P)H : quinone reductase, EC 1.6.99.2) [7] and bacterial oxygen-insensitive nitroreductases [6, 8]. The reactivity of nitroaromatics in single-electron enzymatic reduction increases with an increase in their single-electron reduction potential (E^1_7), and is relatively insensitive to their structure [2–5]. This is consistent with the 'outer-sphere' electron transfer mechanism [9]. In contrast, the mechanism of two-electron reduction of nitroaromatics is less well understood.

Enterobacter cloacae nitroreductase (NR) is a homodimeric 24.5 kDa protein containing an FMN cofactor which reduces nitrofurans, 2,4,6-trinitrotoluene (TNT), and other nitroaromatics [8]. NR follows 'ping-pong' mechanism and reduces nitrobenzene to phenylhydroxylamine in two successive two-electron transfers at the expense of 2 molecules of NADH, the nitroso intermediate being reduced much faster than nitrobenzene [10]. Thus, NR shares the properties of other oxygen-insensitive nitroreductases, such as *Escherichia coli* nitroreductase and DT-diaphorase [6–8]. However, the structure–activity relationships and the mechanism of two-electron (hydride) transfer by *E. cloacae* NR have been insufficiently studied. These studies may be of certain interest, since various strains of *Enterobacter cloacae* are currently being used in biodegradation of nitroaromatic or nitrate ester explosives [11, 12].

In the present work, we have studied the reactions of *E. cloacae* NR with a series of nitrocompounds including high explosives TNT, tetryl, pentryl, TNC, RDX, and HMX (Fig. 1) (cf. Abbreviations). Furthermore, we have established quantitative structure–activ-

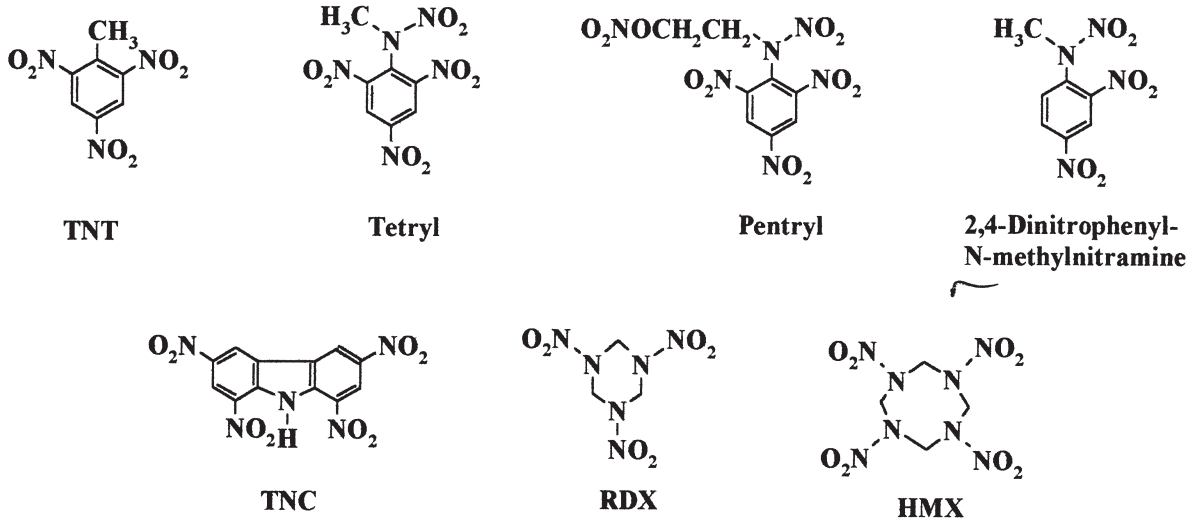

Figure 1. The formulae of nitroaromatic and nitroalicyclic explosives studied in this work.

ity relationships linking the reaction rate with the enthalpies of single- and two-electron reduction of nitroaromatic compounds, obtained by quantum chemical calculations.

MATERIALS AND METHODS

Materials. *Enterobacter cloacae* NR was expressed in *E. coli*, purified and stored as previously described [13]. The enzyme concentration was determined spectrophotometrically using ε_{454} = 14.3 $mM^{-1}cm^{-1}$ for the bound FMN cofactor [13]. The methods of synthesis of nitrocompounds are listed consecutively: TNT, tetryl (2,4,6-trinitrophenyl-*N*-methylnitramine), and 2,4-dinitrophenyl-*N*-methylnitramine [14, 15], pentryl (2,4,6-trinitrophenyl-*N*-nitraminoethylnitrate) [16], TNC (1,3,6,8-tetranitrocarbazole) [17], RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) [18], HMX (octohydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) [19]. The purity of nitrocompounds was determined using melting points, thin-layer chromatography, NMR, IR, and elemental analysis. All other compounds were obtained from Sigma or Aldrich and used as received.

Enzymatic assays and analytical procedures. Kinetic measurements were carried out in 0.1 M Tris/Cl (pH 7.0) containing 0.5 mM desferrioxamine at 25°C. The rate of NR-catalyzed oxidation of NADH by various nitroaromatics was determined by monitoring NADH oxidation ($\Delta\varepsilon_{340}$ = 6.2 $mM^{-1}cm^{-1}$) using a Hitachi-557 spectrophotometer. Corrections were introduced when necessary for the formation of reaction products absorbing at 340 nm. The catalytic constant (k_{cat}) and the bimolecular rate constant (k_{cat}/K_m) of nitrocompound reduction correspond to the reciprocal intercepts and slopes of plots [E]/v *vs.* 1/[$ArNO_2$], where [E] is enzyme concentration, and [$ArNO_2$] is concentration of nitrocompound; k_{cat} is the number of NADH molecules oxidized by a single active center of the enzyme per 1 s. The temperature dependence of k_{cat}/K_m of nitroaromatics was measured between 15–45°C. The reduction of cytochrome *c,* added into the reaction mixture in separate experiments, was monitored spectrophotometrically using $\Delta\varepsilon_{550}$ = 20 $mM^{-1}cm^{-1}$. The rate of oxygen consumption during enzymatic reactions was monitored using a Clark electrode. The concentrations of nitrite were determined spectrophotometrically as described previously [15].

Quantum-mechanical calculations. In semiempirical calculations of compound heat formation (Hf) by the AM1 and PM3 methods, PC Spartan Pro (version 1.0.1, Wavefunction, Inc.) was used. The calculations were performed on nitrocompounds and their single- and two-electron reduced forms specified below. For all calculations, geometries were fully optimized. The enthalpies of reactions (ΔHf) were calculated from Eqns. (1–4), where $ArNO_2$ denotes nitroaromatic compound, $ArNO_2^{-\cdot}$ denotes its anion-radical, ArNO denotes its nitroso derivatie, $ArN(OH)_2$ denotes *N,N*-dihydroxylamine precursor of nitroso derivative [20], and $ArN(OH)O^-$ — its deprotonized form:

a) $1e^-$ transfer: $\Delta Hf(ArNO_2^{-\cdot}) = Hf(ArNO_2^{-\cdot}) - Hf(ArNO_2)$, (1)

b) $2e^-$ (hydride) transfer: $\Delta Hf(ArN(OH)O^-) = Hf(ArN(OH)O^-) - Hf(ArNO_2)$, (2)

or: $\Delta Hf(ArN(OH)_2) = Hf(ArN(OH)_2) - Hf(ArNO_2)$, (3)

or: $\Delta Hf(ArNO) = Hf(ArNO) - Hf(ArNO_2)$. (4)

RESULTS AND DISCUSSION

In accordance with previous observations [10], NR catalyzed the oxidation of 2 moles NADH per mole of mononitrobenzenes. During the NR-catalyzed oxidation of NADH by polynitrobenzenes, the rapid oxidation of 2

NADH equivalents was followed by a second slower phase of oxidation of more than 4 NADH equivalents (data not shown). NR participated in all phases of reaction, since both fast and slow phases were suppressed by 20 μM dicumarol. The first rapid phase of NR-catalyzed NADH oxidation by tetryl, pentryl, TNT or dinitrobenzenes was not accompanied by O_2 uptake or reduction of added cytochrome *c*, or the previously observed nitrite formation, characteristic of the reduction of tetryl and other nitroaromatic *N*-methylnitramines by DT-diaphorase [15]. O_2 was consumed on a time scale of the subsequent phase, with rates and amounts non-stoichiometric to NADH oxidation (not shown). The addition of catalase caused reappearance of oxygen, indicating that H_2O_2 was formed as a final reaction product. The second phase of reaction was also accompanied by the reduction of added cytochrome *c*, which was not inhibited by superoxide dismutase (60 μg/ml) (not shown). Evidently, the hydroxylamine products of reduction of polynitroaromatics were further reduced to dihydroxylamines or other products, which were subsequently reoxidized by O_2 with the formation of H_2O_2 [7, 21], and were able directly to reduce cytochrome *c* [22]. During the second phase of reaction, tetryl, pentryl, and 2,4-dinitrophenyl-*N*-methylnitramine formed a stoichiometric amount of nitrite. The reduction by TNT was not accompanied by nitrite formation. However, characterization of the reaction products is beyond the scope of the present work.

In agreement with the 'ping-pong' scheme for the steady-state kinetics of *E. cloacae* NR [10], we have obtained a series of parallel plots in Lineweaver-Burk coordinates at varied concentrations of tetryl, pentryl or 2,4-dinitrophenyl-*N*-methylnitramine as electron acceptor (10–100 μM) and fixed concentrations of NADH (50–250 μM), (not shown). The values of k_{cat} obtained by extrapolation to an infinite concentration of NADH and the above electron acceptors, and k_{cat}/K_m values of all the electron acceptors investigated are given in Table 1, together with the values of their single-electron reduction potentials (E^1_7). The kinetic parameters refer to the first phase of enzymatic reaction. The k_{cat}/K_m for NADH was almost identical for several electron acceptors used (tetryl, pentryl, and 2,4-dinitro-*N*-methylnitramine), being equal to $(5.9 \pm 0.77)\times 10^6\ M^{-1}s^{-1}$.

The enthalpies ($\Delta H^{\neq}$) and the entropies of activation ($\Delta S^{\neq}$) of nitroaromatics reduction calculated from the temperature dependence of their k_{cat}/K_m values, were following: $\Delta H^{\neq} = 27.04 \pm 1.42$ kJ $\times$ mol^{-1} and $\Delta S^{\neq} = -15.99 \pm 4.77$ J $\times$ mol^{-1} $\times$ K^{-1} (3,5-dinitrobenzoic acid), and $\Delta H^{\neq} = 21.13 \pm 1.38$ kJ $\times$ mol^{-1}and $\Delta S^{\neq} = -58.59 \pm 4.60$ J $\times$ mol^{-1} $\times$ K^{-1} (*p*-nitroacetophenone).

Similar to earlier data [8], the reactivity of *E. cloacae* NR increased upon increase in E^1_7 of several nitroaromatic compounds (Table 1). However, the linear correlation between log k_{cat}/K_m and E^1_7 values was poor, $r^2 = 0.5227$ (not shown), and the data were better described by parabolic approximation ($r^2 = 0.7040$) (Fig. 2). This indicates that the reactivities of nitroaromatics are determined mainly by their electron accepting properties, and not by their particular structure. This served as a starting point for the description of quantitative structure–activity relationships involving compounds with unknown E^1_7 values. It is known that the enthalpies of reactions (ΔHf) obtained by means of quantum-mechanical calculation, exhibit a correlation with single-electron transfer redox potentials [24]. The calculated ΔHf values of nitrocompound single-electron reduction ($\Delta Hf(ArNO_2^{-\cdot})$) are given in Table 2. Further, we have extended this approach to the description of two-electron (hydride) transfer. During reduction of a nitro to a nitroso group, an *N,N*-dihydroxylamine intermediate ($ArN(OH)_2$) is formed [21] (Eqn. 5). It seems unlikely that the formation of $ArN(OH)_2$ with a net $2e^-$, $2H^+$ (or H^-, H^+) transfer, proceeds in a single step. We propose that the initial step

Table 1. Kinetic characteristics of nitrocompound reduction by *Enterobacter cloacae* nitroreductase and single-electron reduction potentials (E^1_7) of nitrocompounds

No.	Compound	k_{cat} (s^{-1})[a]	k_{cat}/K_m ($M^{-1}s^{-1}$)	E^1_7 (V)[b]
1.	Tetryl	800 ± 92 (1820 ± 120)	(7.3 ± 0.51) × 10^6	–
2.	Pentryl	620 ± 75 (1250 ± 115)	(6.3 ± 0.52) × 10^6	–
3.	2,4-Dinitrophenyl-*N*-methylnitramine	410 ± 52 (1020 ± 80)	(1.0 ± 0.15) × 10^7	–
4.	1,3,6,8-Tetranitrocarbazole	116 ± 9.0	(1.5 ± 0.11) × 10^7	–
5.	2,4,6-Trinitrotoluene	143 ± 22	(9.8 ± 1.5) × 10^6	–
6.	2,4-Dinitrotoluene	(660 ± 60)[c]	(2.0 ± 0.06) × 10^{5c}	–
7.	Nifuroxim	47 ± 4.2	(7.8 ± 0.5) × 10^5	–0.255
8.	Nitrofurantoin	102 ± 9.2	(5.0 ± 0.2) × 10^5	–0.255
9.	*p*-Dinitrobenzene	305 ± 15	(3.1 ± 0.2) × 10^6	–0.257
10.	*o*-Dinitrobenzene	91 ± 7.0	(1.46 ± 0.07) × 10^6	–0.287
11.	*p*-Nitrobenzaldehyde	104 ± 8.7	(8.02 ± 0.80) × 10^5	–0.315
12.	*m*-Dinitrobenzene	167 ± 9.0	(5.0 ± 0.23) × 10^5	–0.345
13.	3,5-Dinitrobenzoic acid	323 ± 15	(1.2 ± 0.08) × 10^7	-0.350
14.	3,5-Dinitrobenzamide	370 ± 21	(4.1 ± 0.18) × 10^6	–0.350
15.	*p*-Nitroacetophenone	100 ± 12	(8.05 ± 0.95) × 10^5	–0.355
16.	*o*-Nitrobenzaldehyde	83 ± 7.5	(3.9 ± 0.25) × 10^5	–0.355
17.	*p*-Nitrobenzoic acid	10 ± 1.0	(4.0 ± 0.3) × 10^4	–0.425
18.	*p*-Nitrobenzyl alcohol	20 ± 4.2	(1.0 ± 0.1) × 10^4	–0.477
19.	Nitrobenzene	10 ± 1.3	(3.0 ± 0.33) × 10^4	–0.485
20.	RDX	≤ 0.2	≤ 10^3	–
21.	HMX	≤ 0.2	≤ 10^3	–

[a]NADH concentration, 150 μM. The k_{cat} values in parentheses were obtained at infinite concentrations of both substrates; [b]from ref. [10]; [c]from ref. [23].

in $ArNO_2$ reduction is hydride transfer, yielding an anionic *N,N*-dihydroxylamine form ($ArN(OH)O^-$) (Eqn. 5):

$$ArNO_2 \xrightarrow{H^-} [ArN(OH)O^-] \xrightarrow{H^+} ArN(OH)_2 \xrightarrow[-H_2O]{} ArNO \quad (5).$$

An analogous mechanism has been postulated for the nonenzymatic NADH reduction of quinones, where the rate-limiting net hydride transfer with the formation of anionic hydroquinone (QH^-) is followed by fast protonation (formation of QH_2) [25]. The calculated ΔHf values for all the possible steps in the two-electron reduction pathway (Eqn. 5) are given in Table 2. Irrespective of the favourable energetics of reduction of nitroalicyclic compounds RDX and HMX (Table 2), their reactivities were negligible (Table 1). In spite of the evident specificity of NR to nitroaromatic compounds, the reactivities of RDX and HMX were not used in further structure–activity relationships. The linear correlation between log k_{cat}/K_m of nitroaromatics and ΔHf($ArNO_2^{-\cdot}$) was characterized by r^2 = 0.7120 (PM3), and 0.6958 (AM1); however, the data were better described by a parabolic correlation (r^2 = 0.8106 (PM3) and 0.8495 (AM1, Fig. 3A)). The correlations between log k_{cat}/K_m and ΔHf for a net reaction (ΔHf(ArNO)) were poor: r^2 = 0.1090 (PM3) and 0.2877 (AM1) for a linear approximation, and r^2 = 0.5733 (PM3) and 0.5044 (AM1) for a parabolic approximation (data not shown). The use of ΔHf for hydride transfer and protonation (ΔHf($ArN(OH)_2$)) modestly improved the correlations (r^2 = 0.2990 (PM3)

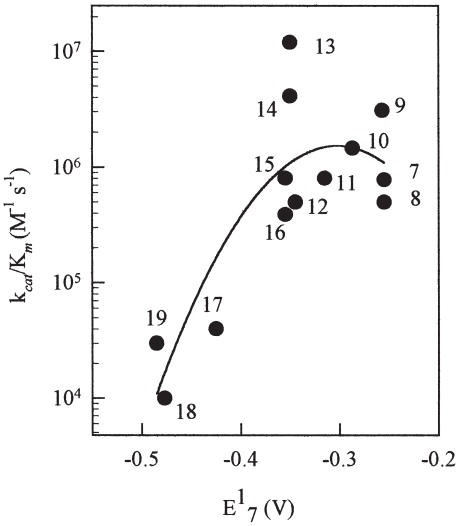

Figure 2. The dependence of log k_{cat}/K_m of nitroaromatic compounds on their single-electron reduction potentials (E^1_7).

The numbers of compounds are taken from Table 1.

and 0.4589 (AM1) for a linear approximation, and r^2 = 0.6469 (PM3) and 0.7185 (AM1) for a parabolic approximation, data not shown). On the other hand, the use of ΔHf for hydride transfer alone ΔHf(ArN(OH)O$^-$) resulted in linear correlations with r^2 = 0.7187 (PM3) and 0.7508 (AM1), and in even better parabolic correlations (r^2 = 0.8106 (PM3) and 0.8495 (AM1), Fig. 3B). Thus, among the calculated ΔHf values for all the possible steps in the two-electron reduction pathway (Eqn. 5, Table 2), the use of ΔHf (ArN(OH)O$^-$) resulted in the best approximation of the quantitative structure–activity relationship (Fig. 3B). This may indicate that the two-electron reduction of nitroaromatics by *E. cloacae* NR proceeds with a rate-limiting hydride transfer and that subsequent steps (i.e., protonation and dehydration, see Eqn. 5) are faster.

One should note that the rates of nitroaromatics reduction by NR depend equally well on both ΔHf of hydride and electron transfer (Fig. 3A, B), and increase upon an increase in their E^1_7 values (Fig. 2). This closely resembles the regularities observed in the reduction of quinones by 1,4-dihydronicotinamides: i) the parabolic reactivity *vs.* E^1 relationship and the transient formation of an ion-radical pair or charge-transfer complex [26] pointed to a multistep (e.g., e^-, H^+, e^-) hydride transfer [26]; ii) the reaction rate increased upon an increase in redox potential of the quinone/anionic hydroquinone (Q/QH$^-$)

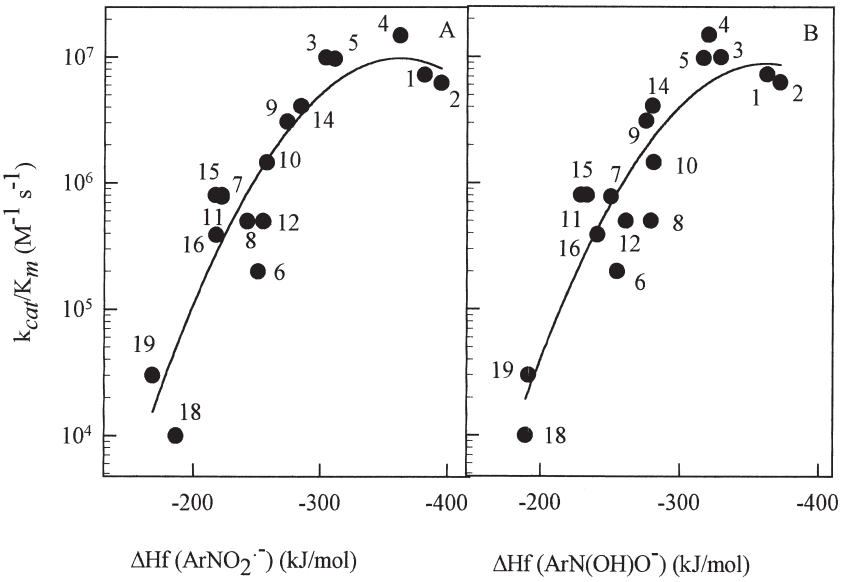

Figure 3. The dependence of log k_{cat}/K_m of nitroaromatic compounds on their enthalpies of single-electron reduction (ΔHf($ArNO_2^{\cdot-}$)) (A), or reduction by hydride ion (ΔHf(ArN(OH)O$^-$)) (B), calculated according to Eqns.1, 2 using AM1 method.

The numbers of compounds are taken from Table 1, ΔHf(ArN(OH)O$^-$) values for trinitrobenzenes correspond to reduction of 4-nitro group.

Table 2. Enthalpies of single- and two-electron reduction of nitroaromatics calculated by Eqns. 1–4 using PM3 and AM1 methods

No.	Compound	ΔHf (kJ/mol)							
		$Hf(ArNO_2^{-\cdot})$		$\Delta Hf(ArN(OH)O^-)$		$\Delta Hf(ArN(OH)_2)$		ΔHf(ArNO)	
		PM3	AM1	PM3	AM1	PM3	AM1	PM3	AM1
1.	Tetryl	−367.77	−381.62	−280.62[a]	−362.50[a]	−62.05[a]	−162.72[a]	88.66[a]	−12.18[a]
2.	Pentryl	−376.48	−394.68	−293.26[a]	−372.21[a]	−58.70[a]	−157.50[a]	88.03[a]	−4.27[a]
3.	2,4-Dinitrophenyl-*N*-methylnitramine	−297.23	−303.76	−229.66[a]	−329.20[a]	−51.46[a]	−142.63[a]	94.73[a]	11.80[a]
4.	1,3,6,8-Tetranitrocarbazole	−367.85	−362.46	−313.05[a]	−320.50[a]	−93.72[a]	−172.42[a]	71.13[a]	−15.15[a]
5.	2,4,6-Trinitrotoluene	−316.44	−310.70	−243.88[a]	−316.77[a]	−53.35[a]	−144.72[a]	90.92[a]	1.13[a]
6.	2,4-Dinitrotoluene	−254.14	−250.58	−182.88[a]	−254.72[a]	−44.93[a]	−134.93[a]	96.86[a]	11.88[a]
7.	Nifuroxim	−227.94	−222.09	−173.55	−250.37	−56.82	−131.80	91.13	−2.22
8.	Nitrofurantoin	−249.95	−242.09	−195.44	−278.95	−56.44	−144.64	92.84	−0.54
9.	*p*-Dinitrobenzene	−281.04	−273.30	−225.14	−275.43	−53.56	−138.53	94.68	12.72
10.	*o*-Dinitrobenzene	−261.00	−257.32	−251.37	−281.04	−96.15	−172.76	30.42	−30.71
11.	*p*-Nitrobenzaldehyde	−223.17	−222.13	−170.45	−232.97	−43.43	−130.21	99.79	17.99
12.	*m*-Dinitrobenzene	−262.13	−254.60	−186.15	−260.91	−46.61	−135.52	95.64	11.09
13.	3,5-Dinitrobenzoic acid[b]	not determined							
14.	3,5-Dinitrobenzamide	−289.16	−284.09	−212.55	−280.24	−50.21	−138.66	93.22	8.58
15.	*p*-Nitroacetophenone	−218.07	−217.07	−163.05	−228.61	−41.21	−129.33	100.46	18.58
16.	*o*-Nitrobenzaldehyde	−207.23	−217.78	−172.21	−240.58	−47.87	−132.72	91.59	15.69
17.	*p*-Nitrobenzoic acid[b]	not determined							
18.	*p*-Nitrobenzyl alcohol	−192.63	−186.36	−140.58	−189.03	−37.61	−124.01	102.38	20.50
19.	Nitrobenzene	−172.09	−167.65	−123.38	−191.13	−36.49	−122.97	102.97	21.30
20.	RDX	−241.42	−250.96	−240.16	−316.90	−60.21	−167.36	66.94	−67.28
21.	HMX	−152.30	−376.81	−20.83	−505.43	−67.99	−145.19	65.06	−77.91

[a]ΔHf for reduction of 4-nitro group of polinitrobenzenes and 1-nitrogroup of tetranitrocarbazole. The ΔHf values for reduction of other nitro groups are more negative by 2–6 kJ/mol. [b]Not determined in view of unavailable pK_a values of free radicals and anionic *N,N*-dihydroxylamine reduction products of nitrobenzoic acids.

couple as well, which was formally consistent with the single-step hydride transfer model [25]. However, the possibility of multistep hydride transfer during reduction of nitroaromatics by NR requires additional lines of evidence, since the values of $\Delta S^{\neq}$ for nitroaromatics reduction obtained in the present work do not entirely favour this mechanism. For comparison, the multistep hydride transfer between dihydronicotinamides and quinones is characterized by a much more negative $\Delta S^{\neq}$ value ($-134\ J \times mol^{-1} \times K^{-1}$ [25]), a consequence of a large negative entropy of the transient charge-transfer complex formation.

CONCLUSIONS

In contrast to the well-documented reactivity *vs.* E^1_7 relationships in single-electron reduction of nitroaromatics by flavoenzymes, quantitative descriptions of two-electron enzymatic reduction are almost absent. The

enthalpies of two-electron (hydride) reduction obtained by quantum chemical calculations in the present work may serve as a useful tool for the analysis of the mechanisms of two-electron reduction, especially of nitroaromatic compounds with presently unknown redox potentials. Although the quantitative structure–activity relationships obtained in this work are specific for a particular enzyme, *E. cloacae* NR is a member of a larger family of proteins including the *E. coli* and *Salmonella typhimurium* nitroreductases, with which it shares over 80% amino acid sequence identity [8,13]. Thus, these results may be extended to related nitroreductases.

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