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QUARTERLY

Electrochemical reduction products of azido nucleosides, including Zidovudine (AZT): mechanisms and relevance to their intracellular metabolism*

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Previous studies on electrochemical reduction of the HIV reverse transcriptase inhibitor, 3′-azido-3′-deoxythymidine (Zidovudine, AZT) and several of its analogues, have been extended to 2′-AZdT and two of the intracellular metabolites of AZT, the 5′-*O*-glucuronide (GAZT) and the 5′-phosphate (AZTMP). Also investigated were azido nucleosides with aglycons susceptible to electrochemical reduction, cytosine and adenine. The surface activities of these compounds at the mercury electrode were examined. In all instances, reduction of the azido group was a two-electron process, with conversion to an amino group. For an azido adenine nucleoside, it proved possible to reduce the azido group without affecting the aglycon. Electrochemical reduction is shown to provide a simple one-step synthesis of amino nucleosides from the available azido nucleosides. The reduced compounds, several hitherto unknown, are useful reference standards for following intracellular metabolism of azido nucleosides, and may also prove of interest as new potential antimetabolites.

The thymidine analogue 3′-azido-3′-deoxythymidine (AZT, Zidovudine, see Table 1), originally synthesized as a potential antitumor agent, but found too cytotoxic for this purpose, was subsequently shown to be a potent *in vitro* inhibitor of the reverse transcriptase of retroviruses, including that of human immunodeficiency virus (HIV), the presumed causative agent of AIDS [1]. Despite some reservations [2], this has led to its widespread use in clinical treatment of AIDS patients and, more recently, also asymptomatic HIV-positive individuals. Such therapy requires monitoring of AZT levels in physiological fluids during treatment, largely by means of h.p.l.c.[1] [3].

Our ongoing studies on electrochemical reduction of purines and pyrimidines, and their nucleosides [4] suggested that electrochemical

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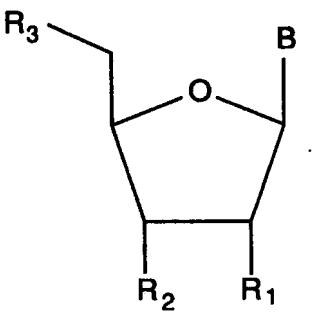
[1]Abbreviations employed: a.c., alternating current; AZT, 3′-azido-3′-deoxythymidine; AMT, 3′-amino-3′-deoxythymidine; 2′-AZdT, 2′-azido-3′-deoxythymidine; AZTMP, AZT-5′-phosphate; AMTMP, AMT-5′-phosphate; GAZT, 5′-*O*-glucuronide of AZT; GAMT, 5′-*O*-glucuronide of AMT; 3′-AZddA, 3′-azido-2′,3′-dideoxyadenosine; 5′-AZ-2′,5′-ddC, 5′-azido-2′,5′-dideoxycytidine; d.c., direct current; h.p.l.c., high performance liquid chromatography; t.l.c., thin-layer chromatography. See Table 1 and Fig. 5 for structures

reduction of AZT might provide a suitable monitoring procedure [5]. It was, in fact, found that such reduction of AZT, and several related analogues (Table 1), proceeds smoothly in aqueous medium, leading to formation of the chemical reduction. The mechanism of reduction is further examined in the light of results for preparative electrolysis, which is also shown to provide a simple one-step synthesis, on a preparative scale, of amino nucleosides

Table 1

Structures and abbreviations of azido nucleoside analogues

Base	Substituent			Compound
B	R_1	R_2	R_3	(abbrevations)
Thymine	H	N_3	OH	AZT
	H	N_3	PO_4^{2-}	AZMP
	H	N_3	G[a]	GAZT
	N_3	H	OH	2′-AZdT
	H	NH_2	OH	AMT
	H	NH_2	PO_4^{2-}	AMTMP
	H	NH_2	G[a]	GAMT
Adenine	H	N_3	OH	3′-AZddA
Cytosine	H	OH	N_3	5′-AZ-2′,5′-ddC



[a]G = β-*O*-glucuronide

corresponding amino congeners. Furthermore, this reduction procedure proved to be sufficiently sensitive for monitoring AZT levels in serum and urine [5, 6].

Interest in AZT as a potent inhibitor of HIV reverse transcriptase has led to the synthesis and study of a large number of nucleoside analogues with azido substituents on the sugar moieties. The availability of such analogues suggested the use of electrochemical reduction for converting these to the corresponding amino analogues, the chemical synthesis of which requires several steps and is both tedious and time-consuming [7 - 9]. Furthermore, pharmacological studies have shown that azido nucleosides undergo partial intracellular reduction to amino nucleosides; in the case of AZT, this leads to formation of 3′-amino-3′-deoxythymidine (AMT) [9, 10], known to be highly cytotoxic to bone marrow cells [8, 11], and undoubtedly one of the sources of the toxic side-effects of AZT.

We now describe extension of the electrochemical reduction procedure to other azido nucleosides (Table 1), including those with base moieties such as adenine and cytosine, which are themselves susceptible to electrochemical reduction, from the now widely available azido nucleosides.

EXPERIMENTAL

We are indebted to Dr. T. Krenitsky (Burroughs-Wellcome, U.S.A.) for a sample of AZT and its 5′-*O*-glucuronide (GAZT); to Dr K. Felczak (Inst. Biochem. Biophys. P.A.S., Warsaw, Poland) for a sample of AZTMP and 5′-AZ-2′,5′-ddC; to Dr. J. Balzarini (Leuven, Belgium) for 2′-AZdT and AMT; and to Dr. N.G. Johansson (Medivir, Stockholm, Sweden) for 3′-AZddA. β-Glucuronidase was from Koch-Light (U.K.).

Direct current (d.c.) polarography was conducted with a Polarographic Analyzer PA 4 (Laboratory Equipment, Praha, Czech Republic). Alternating current (a.c.) polarograms were obtained on a PLP 225C AC-DC Polarograph (COBRABiD, Poland) at a frequency of 120 Hz, amplitude 10 mV. Characteristics of the dropping mercury electrode were: m = 1.65 mg s^{-1}, t = 4.1 s at 70 cm Hg. Curves were recorded at room temperature, and all potentials are relative to the saturated calomel electrode.

Electrochemical reduction was conducted with a Radelkis OH 404 Potentiostat with an Integrator (Budapest, Hungary), in a three-compartment cell, at a constant potential ≤ $E_{1/2}$ of the reduction wave for each compound. The cathode was a mercury pool (12 cm^2) stirred magnetically with a Teflon-coated bar. The reference electrode was a saturated calomel electrode. Progress of electrolysis was monitored polarographically. All solutions were rendered oxygen-free by flushing with pyrogallol-washed argon.

Ultraviolet absorption spectra were run on a Specord M-40 (Zeiss, Jena, Germany). Thin-layer chromatography was carried out using t.l.c. silica gel 60F-254 plates (Merck, Darmstadt, Germany), with solvents: (A) 60% acetone; (B) isopropanol:NH_4OH:H_2O (20:10:5, by vol); and t.l.c. cellulose P-254 plates (Merck, Darmstadt, Germany), with solvents: (C) 1 M ammonium acetate:ethanol (2:5, v/v); (D) isopropanol:NH_4OH:H_2O (7:2:1, by vol).

RESULTS

Direct current polarography

Polarographic data for reduction of the new compounds embraced in the present study, relative to those for AZT analogues previously reported [5], are listed in Table 2. We now discuss each of these in turn.

3'-Azido-3'-deoxythymidine-5'-phosphate. Intracellular phosphorylation of AZT by thymidine kinase leads to AZTMP [12], which is subsequently phosphorylated to the 5'-triphosphate, the inhibitor of HIV-1 reverse transcriptase.

As for AZT [5], AZTMP exhibits, in the pH range 3.5 - 11 (Table 2), a kinetic-diffusion wave ascribed to the azido group, with a polarographic maximum at pH ≥ 8, which disappears at elevated temperatures or in the presence of surface-active compounds (Triton or Et_4NCl).

The half-wave potentials, $E_{1/2}$, are shifted to more negative values relative to those for the parent AZT. The observed breaks in the plot of $E_{1/2}$ *versus* pH at pH 5.3 and 9.8, corresponding to the apparent pK_a values for the azido group and thymidine monoanion formation, are shifted relative to those for AZT (Table 2). The observed break at pH 8.2 is related to the so-called polarographic pK_a' of the azide group. The decrease in value of the limiting current in alkaline medium (Fig. 1) is due to appearance of the thymidine monoanion, not reducible polarographically.

5'-O-Glucuronide-3'-azido-3'-deoxythymidine. In animals and humans AZT is extensively metabolized to its 5'-*O*-glucuronide (GAZT) by UDP-glucuronosyl transferase [11, 15 - 17], and GAZT is readily hydrolyzed by β-glucuronidase to liberate AZT [15], as shown below.

In the pH range 1 - 3.5, GAZT exhibits a single kinetic-diffusion wave (Table 2). It was established, as expected, that glucuronate itself is polarographically inert, so that this wave may be ascribed to reduction of the azido group, as for AZT and its analogues [5]. At pH > 4 there is a polarographic maximum due to adsorption of GAZT; this disappears at elevated temperature or/and in the presence of surfactants, e.g. Triton. A catalytic effect of this compound on hydrogen evolution was also noted (Table 3).

In the pH range 1 - 3 the $E_{1/2}$ was pH-independent, pointing to reduction of protonated GAZT. The breaks in the plot of $E_{1/2}$ *versus* pH (Fig. 1) correspond to the apparent pK_a values for carboxyl (3.0) and azido (4.5) groups, respectively.

Identification of the product of reduction of GAZT was based on its susceptibility to β-glucuronidase. We therefore followed the action of this enzyme on both GAZT (as control) and its reduction product in 0.1 M acetate buffer, pH 4.5, for 30 min at 37°C [18] with monitoring of products by t.l.c. (Table 4). Under conditions where GAZT underwent hydrolysis to liberate AZT, the reduction product was likewise cleaved to yield a single UV-absorbing compound with the R_F of AMT, so that the reduction product was indeed GAMT.

2'-Azido-3'-deoxythymidine. This positional isomer of AZT [19] also exhibits a kinetic-diffusion two-electron reduction wave in the pH range 1 - 13 (Table 2), on which is superimposed a polarographic maximum due to an adsorption-desorption process at pH > 8. As for other azido derivatives, its $E_{1/2}$ is shifted to a more positive potential relative to the 3'-isomer (Table 2), and the $E_{1/2}$ *versus* pH plot exhibits breaks at 5.2 and 9.8 (Fig. 1), corresponding to the apparent pK_a values for the azido group and the ionized thymine ring, respectively.

Table 2

Polarographic data for reduction of azido compounds in aqueous medium at various pH values

Compound	pH range	$-E_{1/2}$/V	Apparent	Diffusion current constant
			pK_a	I_d^a
AZT[b]	1.0 - 4.8	1.020	4.8	5.55
	4.8 - 7.5	0.876 + 0.030 pH		
	7.5 - 9.3	1.105	9.3 (9.5)[c]	
	9.3 - 12.0	0.329 + 0.083 pH		
AZTMP	3.5 - 5.3	1.075	5.3	6.90
	5.3 - 8.2	0.901 + 0.033 pH		
	8.2 - 9.8	1.093 + 0.009 pH	9.8	
	9.8 - 11.0	0.532 + 0.067 pH		
GAZT	1.0 - 3.0	0.920	3.0 (3.5)[c]	5.55
	3.0 - 4.5	0.736 + 0.061 pH	4.5	
	4.5 - 9.5	0.931 + 0.018 pH		
2'-AZdT	1.0 - 2.9	0.838 + 0.042 pH		
	2.9 - 5.2	0.960	5.2	4.92
	5.2 - 9.8	0.836 + 0.024 pH	9.8 (9.5)[c]	
	9.8 - 12.0	0.179 + 0.091 pH		
3'-AZddA	I. 1.0 - 2.2	0.760	2.2 (3.7)[c]	4.01
	2.2 - 3.0	0.650 + 0.050 pH		
	3.0 - 4.0	0.800	4.0	
	4.0 - 6.2	0.544 + 0.064 pH		
	6.2 - 13.0	0.940		
	II. 1.0 - 6.0	1.125 + 0.050 pH		
5'-AZ-2',5'-ddC	I. 1.0 - 3.7	0.890	3.7	3.4
	3.7 - 12.0	0.774 + 0.031 pH		
	II. 3.0 - 5.3	1.122 + 0.039 pH		1.8
	5.3 - 10.3	0.896 + 0.079 pH		

[a] $I_d = (6/7)I_{max}/Cm^{2/3}t^{1/6}$, an approximate value of the faradic n can be obtained by dividing I_d by 2; [b] data from reference [5]; [c] pK_a values for the corresponding non-azido analogues, determined by spectrophotometric titration [13 - 15]

The pH-dependence of $E_{1/2}$ of wave I in the range 1 - 3 (Fig. 1) is due to contamination of the reduction wave for the azido group by the reduction wave of catalytically evolved hydrogen. This is a result of the strong adsorption of the depolarizer in this pH range, and the shift in buffer discharge to more positive potentials (Table 3).

Azido nucleosides with reducible aglycons

Amongst the AZT analogues described previously [5], and above, only the 5-iodouracil derivative underwent simultaneous reduction of the azido group and the C(5)–I bond. In view of the widespread interest in the potential therapeutic activity of azido nucleosides with various purine and pyrimidine aglycons, it is desirable to establish conditions for reduction of such analogues without reduction of the aglycon itself.

Guanine, hypoxanthine and xanthine are not readily susceptible to reduction at the Hg electrode [4], so that the foregoing procedure is applicable to azido nucleosides (and nucleo-

tides, see below) of these bases. By contrast, cytosine and adenine are readily reducible electrochemically [4]. We have therefore sought for conditions under which the azido substituent of cytosine and adenine nucleosides may be reduced with minimal, or no, concomitant reduction of the aglycons, based on the availability of two model compounds, as follows:

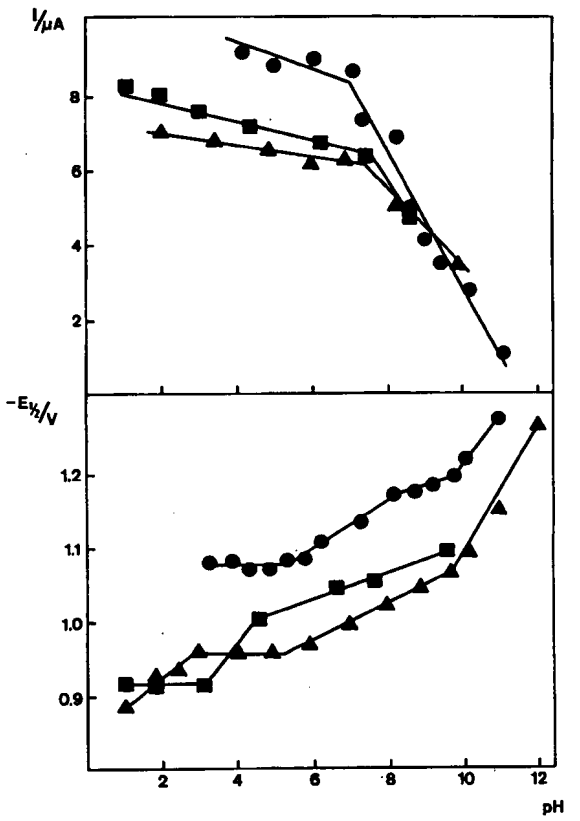


Fig. 1. pH-dependence of half-wave potentials ($E_{1/2}$) and limiting currents (I) for 5×10^{-4} M solutions of: 2'-AZdT (▲), GAZT (■), AZTMP (●)

3'-Azido-2',3'-dideoxyadenosine. This compound exhibits two reduction waves (Table 2): wave I, in the pH range 1 - 13, corresponds to reduction of the azido group; wave II, which appears in the pH range 1 - 6, is associated with reduction of the adenine ring, as for other adenine nucleosides [4, 20]. Both waves exhibit kinetic-diffusion character. For wave I at pH ≥ 8 there is a polarographic maximum due to an adsorption-desorption process of adenine [20].

The observed breaks in the plot of $E_{1/2}$ *versus* pH at 2.2 and 4.0 clearly correspond to the apparent pK_a values for the adenine ring and the azido group, respectively (Table 2). The additional breaks at pH 3.0 and 8.2 then clearly correspond to the so-called polarographic pK_a' values for protonation of the adenine ring and the azido group.

It is clear, from the foregoing, that electrolysis at pH > 6 leads to reduction of the azido group, with the adenine ring left intact, this being further confirmed by the results of macroelectrolysis (see below).

5'-Azido-2',5'-dideoxycytidine. In the pH range 1 - 12 this analogue displays two kinetic-diffusion reduction waves, one (wave I) for the azido group, the other (wave II) for the cytosine ring [4] (Fig. 2). For wave I the break in the $E_{1/2}$ *versus* pH plot at pH 3.7 is appreciably displaced relative to the corresponding apparent pK_a for the azido group of AZT (Table 2). As for the other azido nucleosides, buffer discharge is shifted to more positive potentials (Table 3).

Comparison of the plots of $E_{1/2}$ *versus* pH for waves I and II (Fig. 2) suggests that reduction of only the azido group may be achieved at pH > 6, where its potential for reduction maximally differs by > 0.4 V from that for the cytosine ring.

Table 3

Observed shifts in buffer discharge for azido analogues at 1×10^{-4} M in aqueous medium

Compound	ΔE/mV at various pH values							
	2.0	3.5	4.5	5.5	7.5	8.4	9.5	11.0
AZT	20	70	100	150	200	150	80	40
AZTMP	–	40	60	100	50	20	0	0
GAZT	170	170	180	200	150	50	–	–
2'-AZdT	150	100	50	50	20	0	0	0
3'-AZddA	30	70	180	200	200	200	170	0
5'-AZ-2',5'-ddC	0	20	100	110	180	250	90	–

Table 4
Chromatographic data for GAZT, its reduction product (GAMT), and products of enzymatic hydrolysis

Compound	R_F	
	solvent A	solvent B
AZT	0.91	0.78
AMT	0.56	0.70
GAZT	0.96	0.85
GAZT + β-glucuronidase	0.91	0.78
GAZT reduced (GAMT)	0.80	0.74
GAZT reduced + β-glucuronidase	0.56	0.70

This was, consequently, further examined by means of macroelectrolysis, below.

Alternating current polarography

The results of d.c. polarography described above, pointing to high surface activity (adsorption) of the various analogues, suggested the utility of further examination of this aspect by a.c. polarography, widely applied for this purpose [21].

3'-Azido-3'-deoxythymidine-5'-phosphate. At pH 7.5 and about –0.3 V, there is a shallow depression below the background current (Fig. 3), displaced about 0.2 V relative to the parent AZT [5], ascribed to adsorption of negatively charged species (ionized phosphate) at the site of the positive potential of the electrocapillary maximum [20]. At about –1 V there is a minor desorption peak linked to the peak for AZT reduction. The second adsorption minimum at about –1.7 V testifies to adsorption of the reduction product. This may account for the catalytic hydrogen evolution revealed on d.c. polarograms by the shift in buffer discharge to more positive potentials (Table 3).

Furthemore it should be noted that AZTMP is less strongly adsorbed at the electrode surface than AZT (Fig. 3), as is 5'-AMP relative to adenosine [20], clearly due to the negative charge of the phosphate moiety, with consequent repulsion at the electrode surface.

2'-Azido-3'-deoxythymidine. Like AZT [5], this positional isomer exhibits, at pH 7.5 at about –0.6 V, a marked depression of the current relative to the background current of the

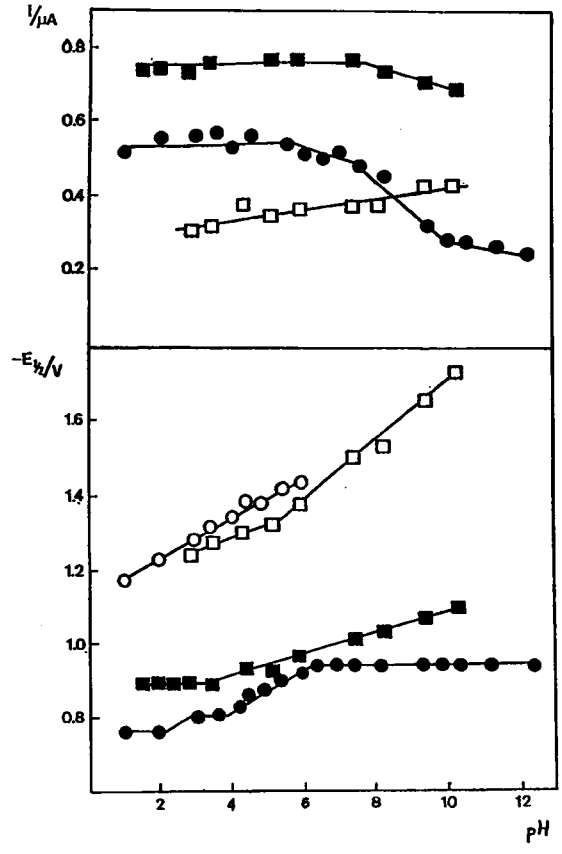


Fig. 2. pH-dependence of half-wave potentials ($E_{1/2}$) and limiting currents (I) for 5 × 10^{-5} M solutions of: 3'-AZddA (●, wave I; ○, wave II), and 5'-AZ-2',5'-ddC (■, wave I; ❑, wave II)

buffer (Fig. 3), testifying to its strong adsorption. In the vicinity of –1 V there is a less pronounced broad reduction peak linked to its desorption peak. In contrast to AZT (Fig. 3), the 2'-isomer exhibits an additional depression at about –1.7 V, corresponding to adsorption of its reduction product.

3'-Azido-2',3'-dideoxyadenosine. At neutral pH this analogue exhibits a depression relative to the background current at about –0.75 V (Fig. 4), due to adsorption. At about –1.1 V there is a peak resulting from the known reorientation of the adenine ring at the electrode surface [20], linked with the peak for reduction of the azido substituent. In acid medium there is an additional peak at about –1.5 V associated with reduction of the adenine ring [20].

5'-Azido-2',5'-dideoxycytidine. At neutral pH this compound shows an adsorption minimum at –0.5 V (Fig. 4) and, like 2'-deoxycytidine [21], no peak for desorption. The peak at –1.2 V

corresponds to reduction of the azido group, and that at –1.6 V (Fig. 4) to reduction of the cytosine ring, as noted above by d.c. polarography.

Macroelectrolysis

Electrolysis was conducted on solutions of AZTMP, GAZT and 2'-AZdT, each at 3×10^{-4} M in 0.1 M phosphate buffer, pH 7.5, at potentials $\leq E_{1/2}$. Polarographic monitoring of the reaction demonstrated disappearance of the reduction wave for the azido group and appearance of an anodic wave for the amino group at $E_{1/2}$ = –0.15 V, as previously observed with AZT [5], as well as with an authentic sample of AMT. The appearance of this anodic wave constitutes further evidence that the products are the amino derivatives. Coulometric measurements for all three compounds pointed to a two-electron reduction, consistent with conversion of the azido to an amino group.

Increasing the potential of electrolysis to $E > E_{1/2}$ led to an increase in the number of electrons (n > 2) involved in the reaction, due to catalytic reduction by H^+ evolved at the electrode surface by adsorbed molecules of the depolarizer. For all three AZT analogues this leads to reduction of the thymine ring, revealed by a decrease in the UV absorption of the electrolyzed solutions. A similar process is observed with guanosine [22], which is adsorbed [21], but not polarographically reducible [4], at the mercury electrode.

For 3'-AZddA at pH 8.2, i.e. under conditions where the adenine ring is not reducible, and at a potential of –1 V, and a concentration of 7×10^{-5} M, electrolysis led to disappearance of the reduction wave of the azido group. Coulometry gave a value of n = 3.3, linked to interference by catalytic hydrogen evolution characteristic for AZT and other azido nucleosides [5], but this was without effect on the adenine ring, as shown by the unchanged UV absorption of the electrolyzed solution.

In the case of 5'-AZ-2',5'-ddC, at 8×10^{-5} M in buffered medium pH ≥ 7, electrolysis at a potential of –1 V led to a 20% reduction of the UV absorption maximum at 271 nm, i.e. the cytosine ring underwent partial reduction as a result of electrolysis, and/or reduction by catalytically evolved hydrogen. It is, consequently, not unexpected that coulometry gave a value of n = 4.2. T.l.c. demonstrated that, whereas the starting compound exhibited R_F values of 0.79 with solvent C, and 0.88 with solvent D, electrolysis led to two products with R_F values of 0.61 and 0.51 in solvent C, and 0.58 and 0.39 in solvent D. The product with lower mobility, and with the same UV absorption spectrum as the starting substance is therefore 5'-amino-2',5'-ddC. The second product is that with the reduced cytosine ring, but no attempt was

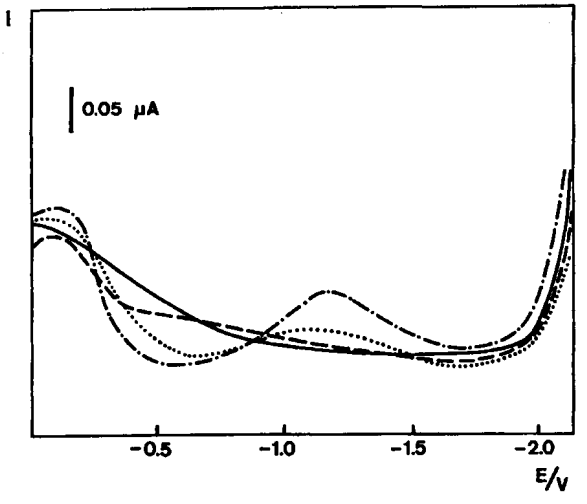


Fig. 3. A.c. polarograms of AZT (–•–), AZTMP (– – –), and 2'-AZdT (• • •) at 8×10^{-5} M in aqueous phosphate buffered medium, pH 7.5. Base current is shown by solid line

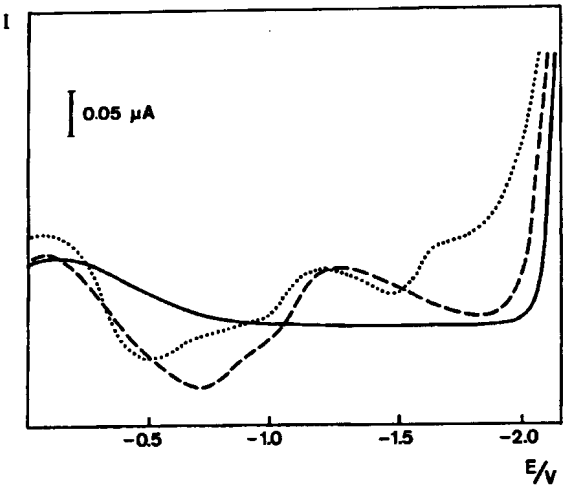


Fig. 4. A.c. polarograms of 3'-AZddA (– – –) at 1×10^{-4} M, and 5'-AZ-2',5'-ddC (• • •) at 5×10^{-4} M in aqueous phosphate buffered medium, pH 7.5. Base current is shown by solid line

made to determine the extent to which the cytosine ring was reduced.

Preparative one-step synthesis of amino nucleosides

The availability of a wide range of azido nucleosides makes possible their conversion to the corresponding amino nucleosides on a preparative scale in a single step by electrochemical reduction. We first illustrate this by synthesis of 3'-amino-2'-deoxythymidine (AMT) from AZT, of which an adequate quantity was available.

A solution of 20 mg AZT in 10 - 20 ml unbuffered water in a 25 ml cell was subjected to electrolysis at a potential of –1.05 V, corresponding to the $E_{1/2}$ for this compound [5]. The course of the reaction was monitored polarographically, and was terminated following disappearance of the reduction wave for the azido group, and appearance of an oxidative anodic wave with $E_{1/2}$ = –0.15 V, corresponding to the presence of an amino group [5]. T.l.c. at this point demonstrated disappearance of the starting AZT. Following removal of water under reduced pressure, the white amorphous residue readily crystallized from anhydrous methanol to give 19 mg (> 95% yield), m.p. 186 - 188°C (uncorr.), as compared to literature values of 187 - 189°C [8] and 187 - 187.5°C [9]. On t.l.c. with several solvent systems the product migrated together with an authentic sample of AMT [5].

Because of availability of an adequate sample of GAZT, an aqueous solution of 30 mg of this compound was subjected to preparative electrolysis, as for AZT, above. The resultant white amorphous product, in virtually quantitative yield, was chromatographically homogeneous. Although difficult to crystallize, its identity was confirmed by hydrolysis with β-glucuronidase to give a product with the R_F of AMT (Table 4).

With the use of larger electrochemical cells, the procedure can be scaled up for larger quantities, as elsewhere demonstrated for the preparation 6-deoxyacyclovir, a pro-drug of the antiherpes agent Acyclovir [23].

The foregoing method is obviously applicable to other azido nucleosides (and nucleotides) with aglycons not susceptible to electrochemical reduction under these conditions, e.g. various 2,4-diketopyrimidines, guanine, hypoxanthine [24], etc.

For azido nucleosides with aglycons such as cytosine or adenine, electrolysis must necessarily be conducted with solutions buffered at appropriate pH values to prevent simultaneous reduction of the aglycon, as described above. In such instances the reduction products may have to be desalted to permit of crystallization. And, even with cytosine nucleosides where partial reduction of the cytosine ring occurs, the desired product of reduction may be isolated by chromatography (see above).

DISCUSSION

The previously proposed two-electron mechanism for electrochemical reduction of the azido group of various AZT analogues [5], leading to formation of the corresponding amino nucleosides and liberation of nitrogen, is now seen to prevail also for the azido nucleoside (and nucleotide) analogues herein reported. A similar two-electron mechanism has been reported for the electrochemical reduction of alkyl [25] and aryl [26] azides on a mercury electrode, for vinyl azides on Hg, Pt and graphite electrodes [27], and for alkyl azides on a modified glassy-carbon electrode [28].

Barone *et al.* [29] independently examined the electrochemical reduction of AZT and concluded, on the basis of chronocoulometric measurements, that the number of electrons involved was n = 4, but made no attempt to identify the product of the reaction. Their higher value of n may have resulted from involvement of reduction by catalytic hydrogen. In our experiments, possible interference by catalytic hydrogen was avoided by conducting electrolysis at potentials $< E_{1/2}$ of the reduction waves. Also of interest, in this context, is the observation of Kuwabata *et al.* [28] that the two-electron reduction of alkyl azides to alkyl amines and nitrogen may be accompanied, but to a much smaller extent at lower concentrations of the depolarizer, by 6-electron and 8-electron steps leading to appearance of hydrazine and ammonia, respectively.

The mechanism of electrochemical reduction of azido nucleosides, and the corresponding reduction products, are of interest in relation to

what is presently known about the intracellular metabolism of AZT [11, 12, 15], depicted in Fig. 5. This does not necessarily exclude other reactions, e.g. intracellular reduction of AZTMP to AMTMP, as observed electrochemically. In addition, whereas the 5′-*O*-glucuronide of AZT appears to be largely excreted in the urine [11], the intracellular fate of its reduction product, AMT-5′-*O*-glucuronide (GAMT), is not known. It could conceivably be as cytotoxic as AMT [11], or undergo cleavage by β-glucuronidase to release cytotoxic AMT.

AZTMP is a moderate inhibitor of the RNase H activity of HIV [30], and a weak inhibitor of cellular thymidylate synthase [31]. Since it is as readily reduced electrochemically as AZT, it may be equally susceptible to intracellular reduction. It would therefore be desirable to examine potential inhibition of the foregoing enzymes by AMTMP. It is also to be expected that the 5′-di- and triphosphates of AZT are equally susceptible to electrochemical reduction, to make available the analogous amino derivatives either on a preparative scale, or as reference compounds for metabolic studies.

The same applies to the numerous azido nucleoside analogues synthesized as potential anti-HIV agents. Electrochemical reduction is a convenient procedure for preparation of their corresponding amino analogues as reference compounds for following intracellular metabolism of the parent azido nucleosides (and their nucleotides), as well as for studies of biological properties of the amino analogues in general, e.g. GAMT, AMTMP, hitherto unknown, as are also the reduction products of the azido cytidine and adenosine analogues.

Mention should also be made of the potential utility of electroreduction for monitoring the level of azido nucleosides in physiological fluids. We have previously described the use of cyclic voltammetry for this purpose, with a lower limit of detection for AZT in aqueous medium of 0.5×10^{-8} M [5]. Independently

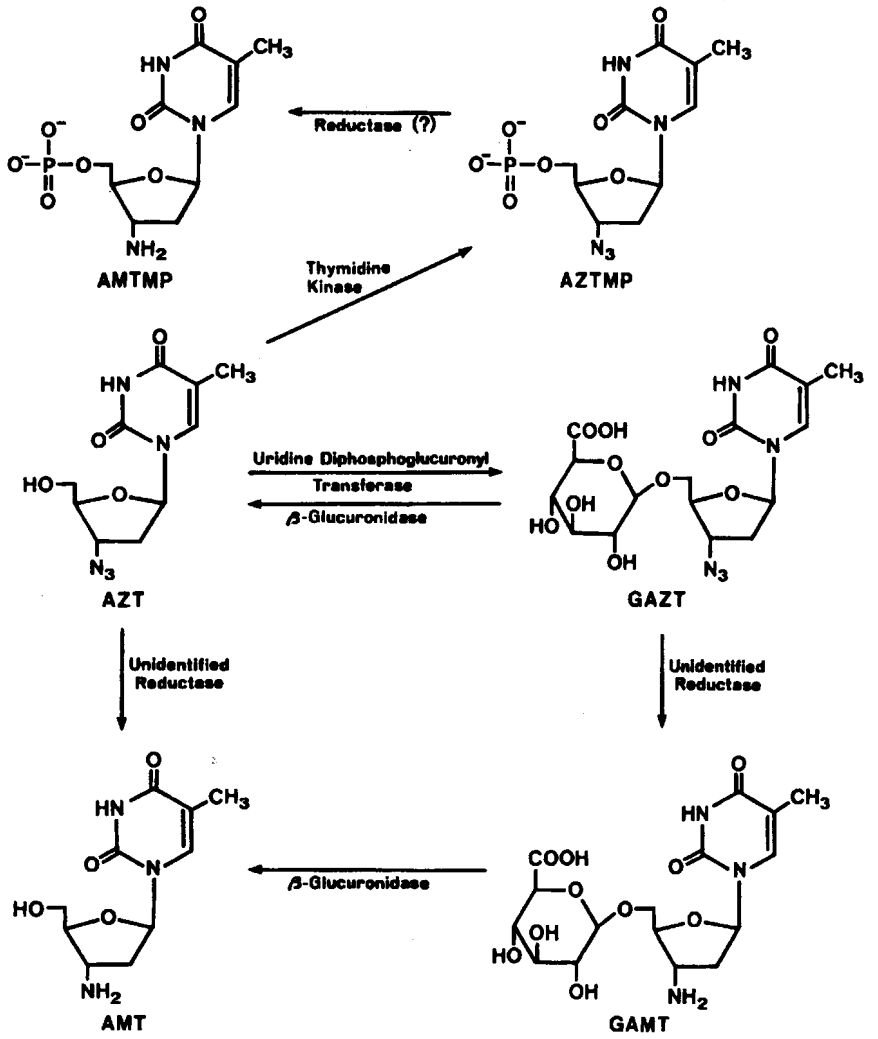


Fig. 5. Proposed metabolic pathways of AZT in humans [11, 12, 15], somewhat modified. The enzymatic conversion of GAMT to AMT is from the present study. It is also possible that AZTMP (normally further phosphorylated to the triphosphate), may be reduced intracellularly to AMTMP, as shown here to occur electrochemically

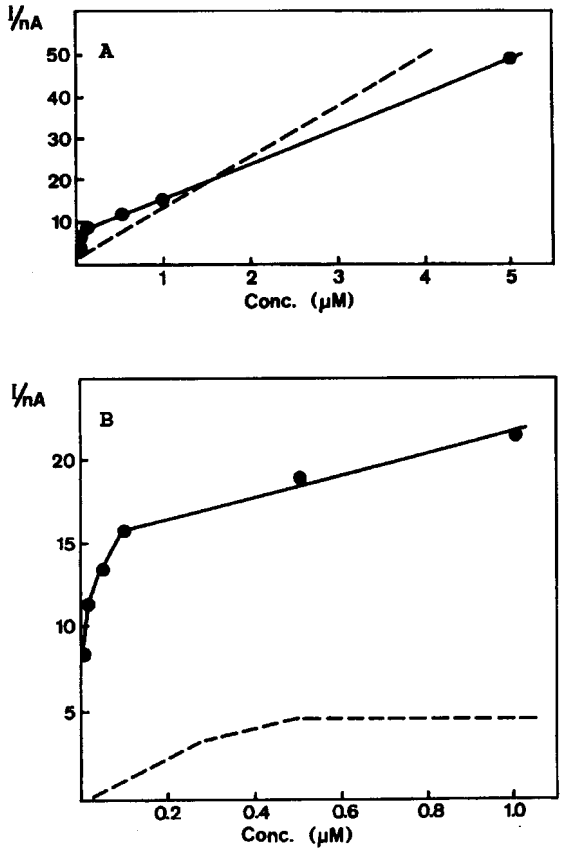


Fig. 6. Comparison of calibration curves for electrochemical monitoring of AZT levels in: (A) aqueous buffered medium, pH 7.5, (B) plasma samples, by cyclic voltammetry (——) [5] and by differential pulse voltammetry (– – –), adapted from Barone et al. [6]

Barone *et al.* [6] reported the use of differential pulse voltammetry for this purpose, with similar sensitivity. Both methods were also applied to monitor AZT levels in serum (and, in our case, also in urine). Comparison of the calibration curves for AZT levels by the two methods (Fig. 6) shows that our procedure is somewhat more sensitive, due largely to use of ethyl acetate extraction of samples prior to voltammetry. The present study shows, however, that neither method distinguishes AZT from AZTMP or GAZT, metabolic products of AZT (Fig. 5), but determines only the overall levels of azido nucleosides and nucleotides. Hence h.p.l.c. would be required to distinguish between these. Nonetheless, the electrochemical techniques are useful monitoring procedures because of their high sensitivity.

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