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Redox turnover of organometallic B_{12} cofactors recycles vitamin C: Sulfur assisted reduction of dehydroascorbic acid by cob(II)alamin

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ABSTRACT

This work reports the reactivity of cob(II)alamin (Cbl(II)) toward reduction of dehydroascorbic (DHA) to ascorbic acid (AA) mediated by sulfur-containing compounds such as glutathione (GSH) and thiocyanate. The reaction supported by GSH proceeded more efficiently than with SCN^-. Our findings demonstrate new aspects of interactions between vitamins B_{12} and C. It has been accepted that simultaneous presence of these vitamins results in their decomposition (viz., irreversible modification of the corrin ring of Cbl and oxidation of AA). We have shown, however, that Cbl(II), the biologically active one-electron reduction product of methyl-Cbl (MeCbl) and adenosyl-Cbl (AdoCbl), is capable of recovering AA in the presence of natural sulfur-containing ligands, within a process that can occur in vivo without glutathione spending, both in a stoichiometric and catalytic manner. Our studies highlight the redox versatility of Cbl(II) and expands the repertoire of reactions whereby redox turnover of the unique B_{12} organometallic cofactors MeCbl and AdoCbl generates Cbl(II), which in turn recycles oxidized vitamin C.

1. Introduction

Dehydroascorbic acid (DHA; Fig. 1A and B) is the product of two-electron oxidation of ascorbic acid (AA; vitamin C; Fig. 1C). Its therapeutic significance attracted a lot of attention, because DHA seems to be the pharmaceutically active form of vitamin C that is preferentially imported by colorectal cancer cells and is responsible for selective toxicity of vitamin C to tumor cells [1]. Upon cell uptake it is reduced to AA, predominantly by glutathione (GSH; Fig. 1D), thioredoxin and NADPH [2–5], which represents an important mechanism of its biological and therapeutic actions [1]. The question remains, whether DHA reduction is always related to GSH consumption or if it can proceed through other pathway, e.g. involving metal centers. The only example of DHA reduction by metal complexes relates to the reaction with iron-nitrosyl-hemoglobin leading to the formation of methemoglobin and NO liberation [6].

Cobalamin (B_{12}, Cbl) is an essential micronutrient required by all cells in the body. An important fraction of the intracellular pool of cobalamin, the only metal-containing vitamin (Fig. 1E), is its one-electron reduced form, i.e. cob(II)alamin (Cbl(II)) [7–9], which can be considered as a reducing agent. The vast majority of cob(II)alamin present in biological systems derives from the one-electron reduction of MeCbl and AdoCbl, as part of the catalytic cycle of cognate enzymes methionine synthase and methylmalonyl-CoA mutase [8,9]. Chemically, the reducing activity of Cbl(II) was observed in the reactions with oxidizing free radicals, e.g. superoxide (O_2^-) [10,11], nitric oxide (NO) [12–14], nitrogen dioxide (NO_2) [15,16] and chlorine dioxide (ClO_2) [17]. Both Cbl(III) and Cbl(II) form complexes with GSH and SCN^- that are abundant compounds in nature [18–23], with concentrations of GSH reaching values of 10 mM in cells [24] and those for SCN^- being 0.5–6.0 mM in mucosal liquids and ca. 10–100-fold lesser in blood [25,26]. In cells, cobalamins are present in relatively low concentrations

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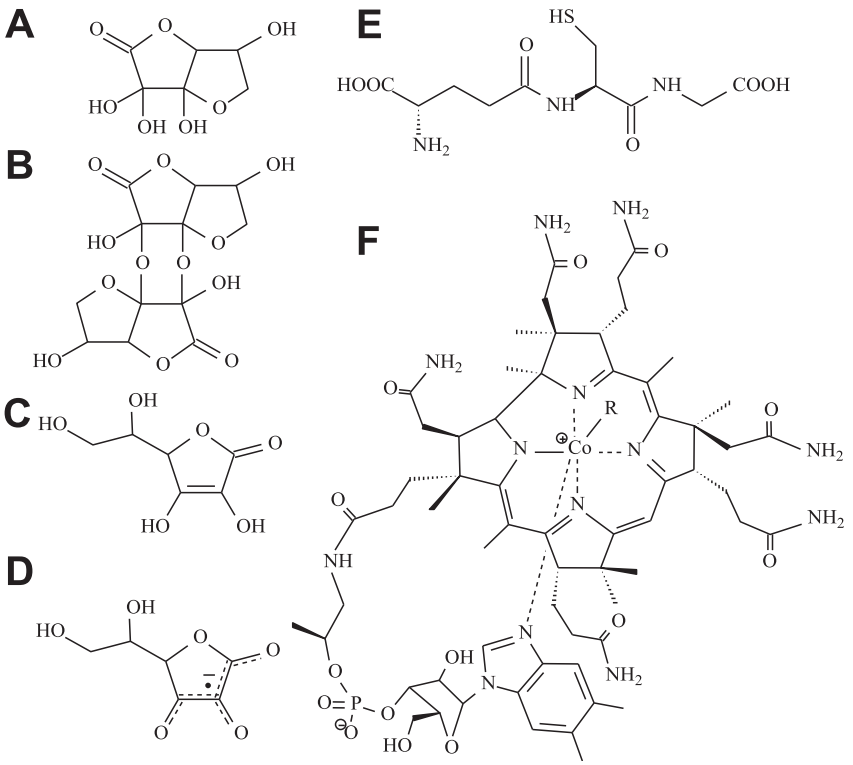

Fig. 1. Structures of (A) monomeric, MDHA, and (B) dimeric, DDHA, forms of dehydroascorbic acid, (C) ascorbic acid, (D) ascorbyl free radical, (E) glutathione and (F) cobalamin ($R = H_2O$, CN^-).

(0.15–0.20 nM), but their reduced forms cob(II)alamin and cob(I)alamin are highly reactive [27]. Glutathionylcob(III)alamin (GS^--Cbl(III)) was found in vivo [27]. Important characteristic of these complexes is the existence of a Co–S bond, strengthening the notion that Co–S bonded cobalamins, including the fraction of SCN^- that binds through the sulfur atom partake in the complex map of cellular cobalamin species [20,21].

Knowing that extra-ligands may influence redox behavior of the metal centers we were interested to reveal the reactivity of Cbl(II)/GSH-system toward DHA. Therefore, in this work we report kinetic and mechanistic studies of reaction of Cbl(II) with dehydroascorbic acid in acidic and neutral medium (at room temperature) in the presence of glutathione. For comparison, the studies were performed with thiocyanate as well, which differs from GSH in that it is a redox inactive (under applied experimental conditions) S-donating ligand. Interestingly, we have observed a catalytic effect of S-containing ligands on the reduction of DHA by Cbl(II). This has important mechanistic implications related to physiological and therapeutic aspects of vitamin C: (1) Cbl(II) formed during normal redox cycling of organometallic derivatives MeCbl and AdoCbl can recycle vitamin C in a process assisted by natural sulfur-containing species, and (2) the intracellular conversion of DHA to vitamin C mediated by Cbl(II) does not occur at the expense of glutathione consumption.

2. Experimental

2.1. Chemicals

Hydroxocobalamin hydrochloride (Fluka; ≥ 95%), dehydro-L-(+)-ascorbic acid dimer (Sigma-Aldrich; ≥ 80%), sodium borohydride (Aldrich; ≥ 96%), glutathione (Sigma-Aldrich; ≥ 98%), potassium thiocyanate (Sigma-Aldrich; ≥ 99%) were used as received. Concentrations of Cbl were found by means of conversion to dicyano form ($\varepsilon_{367} = 30{,}400\ M^{-1}\ cm^{-1}$) [28]. Cbl(II) was prepared using sodium borohydride according to a published procedure [29]. Excess borohydride was destroyed by adding acetone. Stock solutions of DHA were prepared by dissolving the commercial compound in diluted H_3PO_4 (pH 2.3), kept at 5 °C and used within 3 h. Concentrations of DHA were determined after its reduction to AA ([GSH] = 50 mM, pH 5–6) using published extinction coefficients [30]. Air-free argon was used to deoxygenate solutions. Phosphoric acid, mono- and disubstituted potassium phosphate and sodium acetate were used to adjust pH. All experiments were performed in 0.1 M buffers under anaerobic conditions.

2.2. Methods

UV–vis spectra were recorded on thermostated (±0.1 °C) Cary 50 spectrophotometer in gas-tight quartz cells with optical pathlength 1.00 cm. Stopped-flow experiments were performed on μSFM-20 BioLogic device equipped with J&D TIDAS detector.

Cyclic voltammetry (CV) experiments were carried out using an Autolab instrument with a PGSTAT 30 potentiostat. A conventional three electrode arrangement was employed consisting of a glassy carbon working electrode (Metrohm), a platinum wire as the counter electrode and silver chloride (in 3 M NaCl) reference electrode (0.222 V vs NHE at 20 °C). Prior to the measurements, the surface of the glass-carbon electrode was polished with alumina powder. Lithium perchlorate (0.1 M) was used as the supporting electrolyte.

Analysis of reaction products was performed anaerobically using water as a solvent (the resulting pH of the samples was 5.3). Starting material, adducts and products were identified by cryo-

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