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PHYSICAL CHEMISTRY OF SOLUTIONS

Kinetics of the Reaction between Cobinamide and Isoniazid in Aqueous Solutions

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Abstract—The kinetics and mechanism of the reaction of diaquacobinamide (Cbi(III)) with isoniazid (isonicotinoyl hydrazide (INH)) are studied. It is determined that the composition of the products depends on the ratio between the concentrations of the reactants. Adding excess INH to cobinamide results in the rapid formation of a stable complex of Cbi(III) with two isoniazid molecules. If the concentrations of isoniazid and cobinamide are close, or cobinamide is in excess, then a complex of Cbi(III) with one isoniazid molecule initially forms. There is then a fast inner-sphere electron transfer to yield an unstable complex of reduced Cbi(II) with hydrazyl radical (RN_2H_2)(Cbi(II)) that decomposes to form reduced cobinamide and the products of the oxidation of isoniazid: isonicotinamide, pyridine-4-carboxaldehyde, and isonicotinic acid (INA). It is concluded that with a 1000% excess of cobinamide, the main product of the oxidation of INH is INA.

Keywords: kinetics, reaction mechanism, antidote, cobinamide, isoniazid

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INTRODUCTION

Poisoning with toxic alcohols, cyanide, hydrogen sulfide, and carbon monoxide, along with intentional or unintentional overdoses of medications, are the second leading cause of injury-related morbidity and mortality [1, 2]. One way of preventing poisoning is to use special substances (antidotes) that can reduce or eliminate the effects of poisons on the human body. There are several mechanisms behind the action of antidotes: (1) interacting with poisons to form nontoxic substances, (2) accelerating the removal of poisons from the body, (3) reducing the rate of the transformation of poison into more toxic substances, (4) competing with the poison for essential receptor sites, and (5) blocking essential receptor sites that enhance the toxic effect of the poison [1, 3].

Isoniazid (isonicotinoyl hydrazide (INH)) (Fig. 1a) is a highly efficient antituberculosis drug [4, 5] on the list of vital and essential medicines. Among clinical issues in using INH are its neuro- and hepatotoxicity [4, 5]. An overdose of isoniazid adversely affects the human body [6]. It is recommended that pyridoxal (vitamin B_6) be used to reduce its toxic effect [7]. However, there are literature data that testify to the ineffectiveness of using pyridoxal to treat isoniazid overdoses [8].

Cobinamide (Fig. 1b) is a precursor of vitamin B_{12} (cobalamin (Cbl)) during its biosynthesis [9]. Cobinamide differs from vitamin B_{12} by the absence of a ribonucleotide fragment. It has been determined that diaquacobinamide is a more efficient antidote for cyanide [10, 11] and hydrogen sulfide [12–15] poisoning than aquacobalamin, and it is an efficient NO scavenger [16].

In [17], we studied the interaction between vitamin B_{12} and isoniazid. It was shown that during the reaction cobalamin reversibly binds with the neutral INH molecule. However, we failed to find information on the interaction between isoniazid and cobinamide.

The aim of this work was to find the kinetic parameters and mechanism behind the reaction between isoniazid and cobinamide.

EXPERIMENTAL

Isoniazid (>98%), isonicotinic acid (99%), and isonicotinamide (99%) (Alfa Aesar) were used in our experiments. Diaquacobinamide was synthesized according to the procedure in [18]. The other reagents were of chemically pure grade.

The products of the reaction of isoniazid with cobinamide (Cbi(III)) in a neutral medium were determined as follows. An aqueous isoniazid solution was added to an aqueous cobinamide solution under anaerobic conditions. The Cbi(III)-to-INH ratio in the obtained solution was 1 : 2 and 4 : 1. This solution was kept for several hours until the completion of the reaction. The solution was then passed through a silica gel (1 g) column and eluted with 100 mL of distilled water. Cobinamide was adsorbed and retained by silica gel, while isoniazid and the products of its oxidation

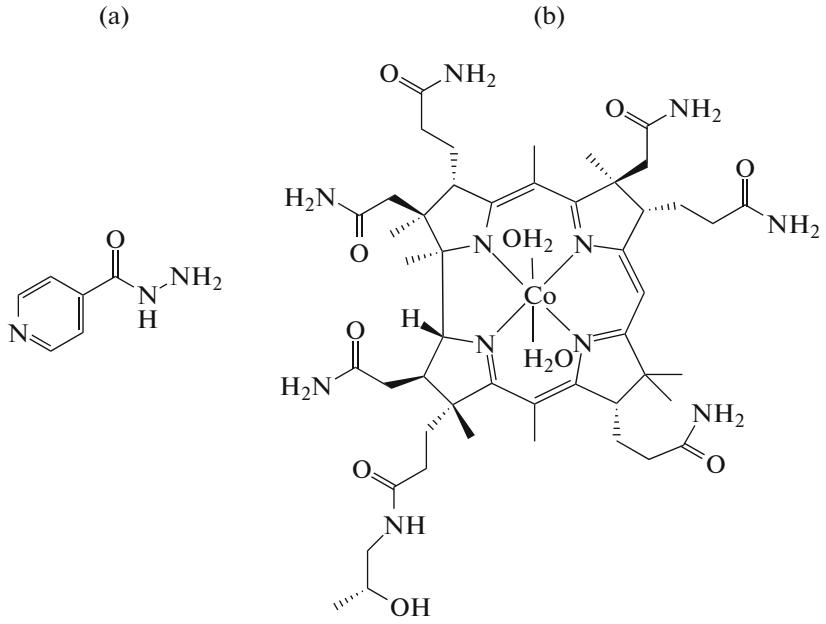


Fig. 1. Structures of (a) isoniazid and (b) diaquacobinamide.

were eluted from the column. The aqueous solution passed through the chromatographic column and was evaporated in vacuum at 40°C until a dry residue formed. The residue was dissolved in ethanol (95 vol %) and analyzed via gas chromatography–mass spectrometry on a Shimadzu GCMS-QP2010 Ultra gas chromatograph–mass spectrometer using a Zebron ZB-5ms capillary column. The mobile phase was helium (30 mL/min). The column temperature (120°C) was held constant using a thermostat.

Kinetic studies were performed under anaerobic conditions with a Varian Cary 50 spectrophotometer using a 1-cm sealed quartz cell. Fast reactions were studied using an RX2000 rapid mixing stopped-flow unit.

The anaerobic conditions were created using argon.

The rate of the reaction between cobinamide and isoniazid was determined from the results of measuring the absorbance at wavelengths of 412, 460, 520, and 537 nm.

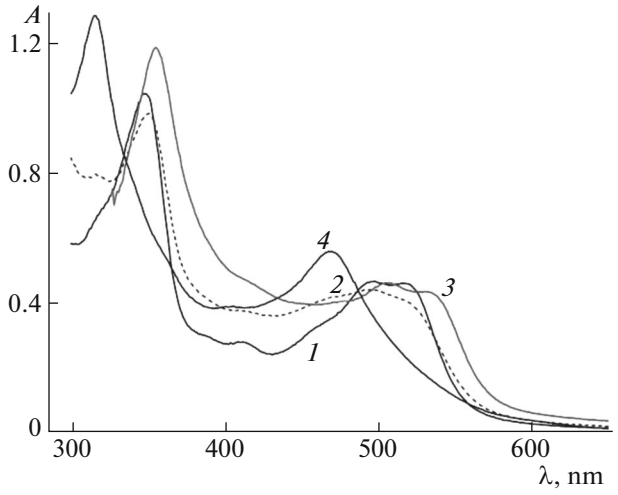


Fig. 2. UV–Vis spectra recorded during the reaction between cobinamide and isoniazid at pH 7.5: (*1*) initial cobinamide, (*2, 3*) intermediates, and (*4*) final product; $[Cbi(III)]_0 = 5 \times 10^{-5}$ M; $[INH]_0$ = (*2*) 1 and (*3*) 50 mM at 25°C under anaerobic conditions.

RESULTS AND DISCUSSION

It was found that adding excess isoniazid to an aqueous cobinamide solution changes the color of the solution from red to yellow in the pH range of 4–13.

Analysis of the UV–Vis spectra recorded during the reaction of cobinamide with isoniazid at pH ≥7.5 revealed two successive events (Fig. 2).

The first event was very fast and depended on the isoniazid concentration. At INH concentrations of <1 mM, the peaks of the initial cobinamide at 348, 495, and 517 nm vanished (Fig. 2, spectrum *1*), and new peaks emerged at 350 and 497 nm (Fig. 2, spectrum *2*). At high (>5 mM) isoniazid concentrations, peaks at 355, 507, and 532 nm emerge (Fig. 2, spectrum *3*). Such changes are characteristic of the reactions of complexation of cobinamide with different ligands (L): thiocyanate [19], cyanamide [20], hydrogen sulfide [13], and cyanide [21]. In these reactions, coordinated water molecules are successively substituted to form LCbi(III) and $(L)_2$Cbi(III).

The second event was accompanied by the vanishing of the peaks of the first intermediate and the emergence of new peaks at 315 and 469 nm (Fig. 2, spectrum *4*). The final spectrum of the reaction product

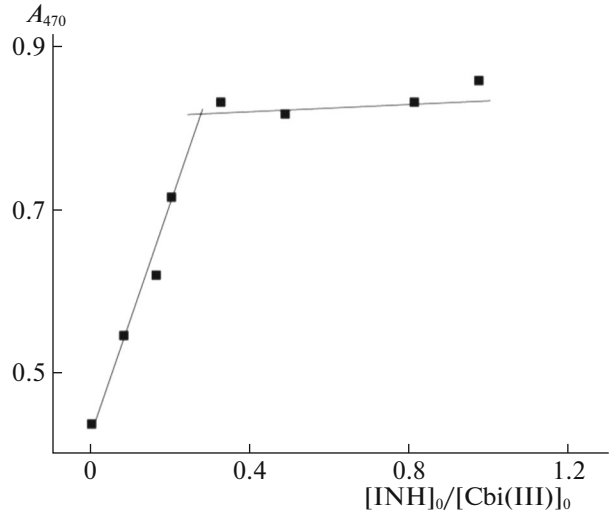


Fig. 3. Spectrophotometric titration of cobinamide with isoniazid at pH 7.5.

corresponded to the reduced form of cobinamide Co^{2+} (Cbi(II)) [13, 22].

When pH is reduced to 4.5, the reaction between isoniazid and cobinamide proceeds in one step and is accompanied by the vanishing of the peaks of the initial cobinamide (349 and 520 nm) and the emergence of new peaks (315 and 469 nm) corresponding to Cbi(II).

This shows that cobinamide acts as an oxidant of isoniazid over a wide range of pH.

Kinetic Studies

We failed to study the kinetics of the reaction of complexation of cobinamide with isoniazid because this reaction is very fast and finishes in the time it takes to mix the reagents throughout the investigated pH range.

Figure 4 shows typical kinetic curves of cobinamide reduction.

Overall Stoichiometry of the Reaction

Figure 3 presents the results from spectrophotometric titration of cobinamide with isoniazid under anaerobic conditions.

It was found that the full reduction of Cbi(III) was attained at [IHN] : [Cbi(III)] = 1 : 4.

Note that in redox reactions, isoniazid acts as a one- or four-electron reducer [23, 24].

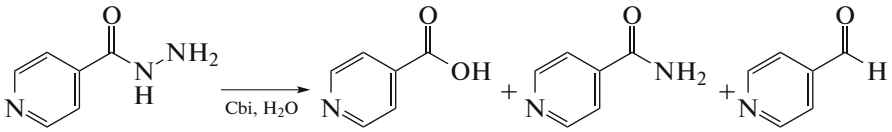
Determining Products of the Reaction between Isoniazid and Cobinamide in a Neutral Medium

Gas chromatography–mass spectrometry was used to determine the products of the reaction of isoniazid with cobinamide in a neutral medium: isonicotinic acid, isonicotinamide, and pyridine-4-carboxaldehyde (Table 1). The initial isoniazid was not found in the studied sample, indicating the isoniazid was fully oxidized.

From the areas under the peaks, it was found that at [Cbi(III)] : [IHN] = 1 : 2, the main product is isonicotinamide (68%, Table 1); at [Cbi(III)] : [IHN] = 4 : 1, it is isonicotinic acid (90%, Table 1).

Similar changes were observed in the oxidation of isoniazid by catalase peroxidase from *Mycobacterium tuberculosis* [25].

The overall scheme of the oxidation of isoniazid by cobinamide in aqueous solutions under anaerobic conditions at pH 7.5 can thus be presented as



(1)

It is seen from Fig. 4 that the initial absorbance grows along with the isoniazid concentration. This is explained by the formation of a complex of cobinamide with two INH molecules (Fig. 2). It was also found that the drop in the absorbance (which corresponds to the accumulation of the reduced form of cobinamide (Cbi(II))) slows as the excess of isoniazid with respect to cobinamide grows.

Table 1. Characteristics of the products of the reaction

Substance	R_t, min	m/z	Yield, %	
			[Cbi(III)] : [INH] = 1 : 2	[Cbi(III)] : [INH] = 4 : 1
Pyridine-4-carboxaldehyde	2.170	107	16	–
Isonicotinamide	3.537	122	68	10
Isonicotinic acid	4.346	123	16	90
Isoniazid	16.257	137	—	—

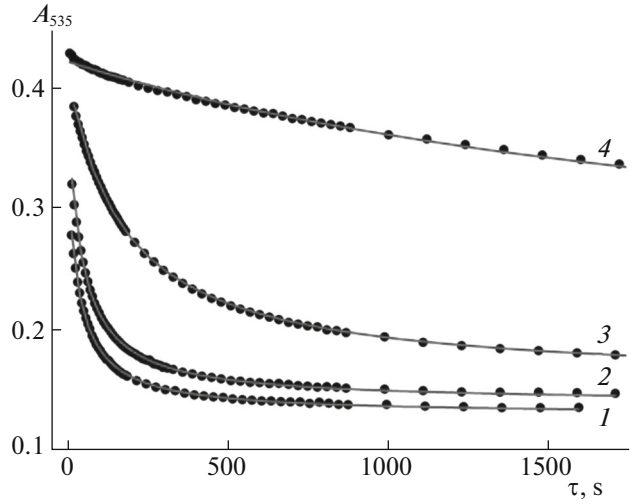


Fig. 4. Kinetic curves of the reduction of cobinamide with an excess of isoniazid: $[Cbi(III)]_0 = 5 \times 10^{-5}$ M; $[INH]_0$ = (*1*) 0.25, (*2*) 1, (*3*) 5, and (*4*) 50 mM at 25°C and pH 7.5 under anaerobic conditions.

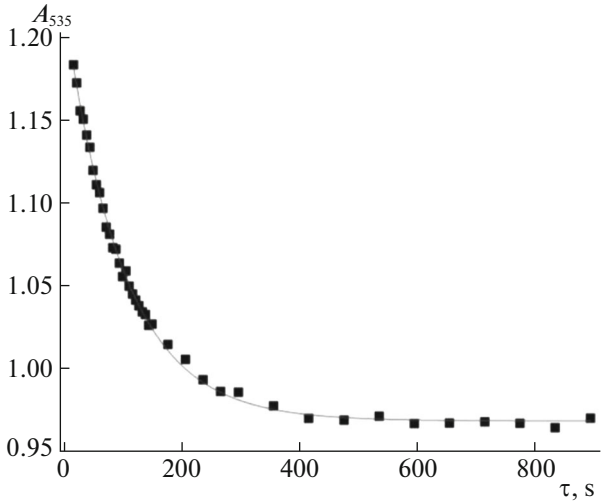


Fig. 5. Typical kinetic curve of the reduction of cobinamide by isoniazid with a deficiency of the latter (second step): $[Cbi(III)]_0 = 1.5 \times 10^{-4}$ M; $[INH]_0 = 1.25 \times 10^{-5}$ M at 25°C and pH 7.5 under anaerobic conditions.

The kinetic curves were linearized in coordinates of $(A_0 - A_t)/(A_f - A_t)$ versus time and are described by the equation

$$A_t = \frac{A_0 + ktA_f[Cbi(III)]_0}{1 + kt[Cbi(III)]_0}, \quad (2)$$

where A_0, A_t, and A_f are the initial, current, and final absorbances, respectively; $[Cbi(III)]_0$ is the total cobinamide concentration in the reaction, M; k is the second-order reaction rate constant; and t is time, suggesting that the order of the reaction with respect to cobinamide is 2 (k_{obs2}, $M^{-1}\,s^{-1}$) [26].

The dependence of k_{obs2} on the isoniazid concentration at pH 7.5 is nonlinear; k_{obs2} falls as the isoniazid concentration grows (Table 2).

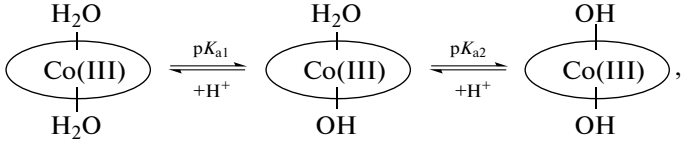
The drop in the rate of the cobinamide reduction reactions upon a simultaneous increase in the concentration of the complex of cobinamide(III) with two isoniazid molecules shows that adding two INH molecules to Cbi(III) stabilizes the oxidation state of Co(III) and prevents reduction. Note that similar results were obtained for the interaction between Cbi(III) and hydrogen sulfide [13].

Figure 5 shows a typical kinetic curve of the oxidation of INH by an excess of Cbi(III). Processing of the kinetic data in semilog coordinates showed that the partial order of the reaction was 1 (k'_{obs2}, s^{-1}).

The dependence of the observed rate constant for the oxidation of INH on the Cbi(III) concentration is nonlinear, but it can be linearized in coordinates of k'_{obs2} versus $[Cbi(III)]^2$. This indicates the order of the reaction with respect to cobinamide was 2. Linear fitting of this dependence allowed us to calculate third-order constant $k' = (4.55 \pm 0.2) \times 10^5\ M^{-2}\,s^{-1}$ at pH 7.5 and 25°C under anaerobic conditions.

Our kinetic studies thus demonstrated that two Cbi(III) molecules are required for the formation of reduced cobinamide, and the reaction is described by the kinetic equation

$$k_{obs2} = k'[Cbi(III)]^2, \quad (3)$$



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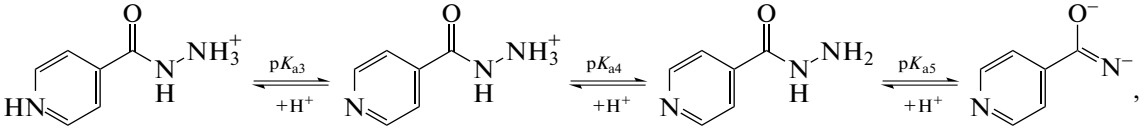

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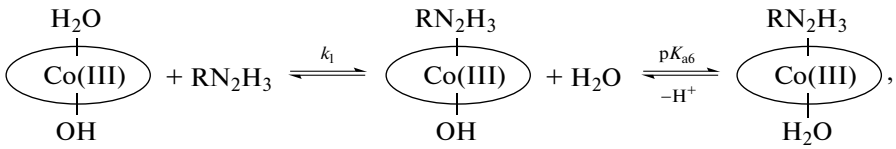

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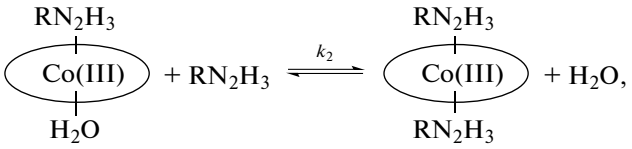

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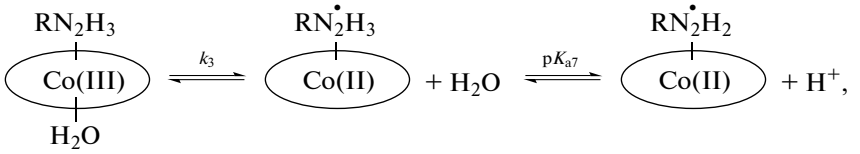

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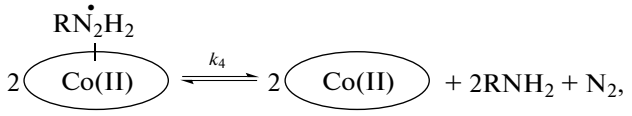

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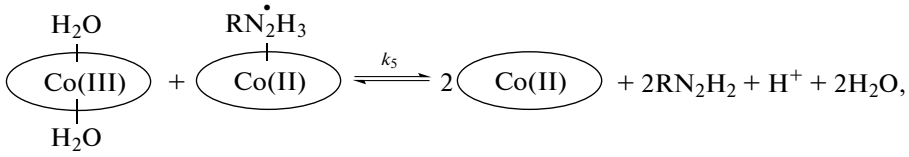

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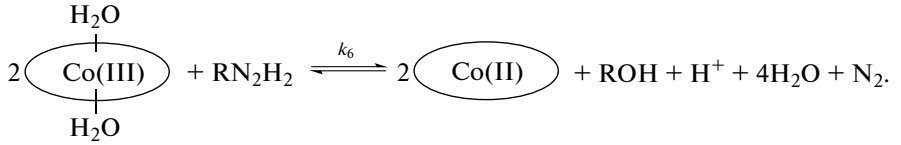

(11)

In an aqueous solution, cobinamide exists as diaquacobinamide $(H_2O)_2$Cbi(III), aquahydroxocobinamide $(H_2O)(OH^-)$Cbi(III), and the dihydroxo form $(HO^-)_2$Cbi(III) with $pK_{a1} = 5.9$ and $pK_{a2} = 10.2$ (reaction (4)) [13]. Note that only diaquacobinamide and aquahydroxocobinamide participate in the complexation reactions, since the hydroxide ion is inert to substitution in the inner sphere of the complex.

INH also has acid–base properties and exists in the solution in four forms (reaction (5)) with $pK_{a3} = 1.99$, $pK_{a4} = 3.67$, and $pK_{a5} = 10.89$ [23]. At physiological pH values, isoniazid is primarily in the neutral form (RN_2H_3, where R is acyl of isonicotinic acid).

Table 2. Dependence of k_{obs2} on the isoniazid concentration (pH 7.5, 25°C; anaerobic conditions)

[INH], mM	k_{obs2}, $M^{-1}\,s^{-1}$
0.25	659
1	546
5	119
50	7.8

Based on the data on the products and stoichiometry of the reaction and the results from kinetic studies, we can propose the following mechanism of the information of isoniazid with cobinamide in a neutral medium:

This mechanism includes reversible steps of sequential binding of two molecules of isoniazid (RN_2H_3) by cobinamide (reactions (6) and (7)). The complex with one isoniazid molecule is unstable; there is an electron transfer to form a complex of reduced Cbi(II) with hydrazyl radical (reaction (8)). The bonding of the second isoniazid molecule (reaction (7)) prevents reaction (8) and stabilizes the 3+ oxidation state of cobalt in cobinamide. The formation of the complex of cobinamide with two isoniazid molecules shows that the main form produced after adding the first INH molecule is $(RN_2H_3)(H_2O)$Cbi(III), since OH^- is inert to substitution.

The complex (RN_2H_2)(Cbi(II)) of reduced Cbi(II) with the hydrazyl radical is unstable and decomposes. The difference between the reaction products suggests this process proceeds through two mechanisms. The first is the disproportionation of this complex to form two Cbi(II) molecules, isonicotinamide (RNH_2), and

nitrogen (reaction (9)). The second is the oxidation of complex (RN_2H_2)(Cbi(II)) by a second Cbi(III) molecule to form two Cbi(II) molecules and the product of the two-electron oxidation of isoniazid (reaction (10)). In both cases, the reaction order with respect to cobinamide is two because the rate-determining step involves two cobinamide molecules.

The product of the two-electron oxidation of isoniazid (RN_2H_2) is unstable and rapidly oxidized to isonicotinic acid (ROH) and nitrogen by an excess of cobinamide (reaction (11)).

CONCLUSIONS

It was shown in this work that at near-physiological pH values, cobinamide can rapidly form a complex with one or two isoniazid molecules. The binding of isoniazid to cobinamide under the same conditions (pH 7.5, 25°C) is approximately 300 times faster than the binding by aquacobalamin (vitamin B_{12}) [17]. The complex of Cbi(III) with one isoniazid molecule (which is formed primarily when the isoniazid and cobinamide concentrations are close, or when cobinamide is in excess) is unstable and forms reduced cobinamide and products of the oxidation of isoniazid: isonicotinic acid, isonicotinamide, and pyridine-4-carboxaldehyde. The main product of the oxidation of INH by an excess of cobinamide is isonicotinic acid, which is less toxic than INH. The formation of INA also testifies to the ability of cobinamide to oxidize active metabolites (radical forms) of INH, which form via the oxidation of INH and inactivate biological substances [27–29].

Unlike cobinamide, aquacobalamin cannot oxidize INH [17].

The complex of cobinamide with two INH molecules (which forms in a large excess of isoniazid) is stable at pH 7.5 and 25°C; there is no oxidation of isoniazid.

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