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Reactions of Cobinamide with Glucose and Fructose

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Kinetics of cobinamide (Cbi) reduction by glucose and fructose was studied at alkaline solutions. It was shown that the reaction of cobinamide with monosaccharides leads to the formation of Co(I) complex in contrast to the reduction of aquacobalamin (H_2OCbl) resulting in the formation of Cbl(II).

Keywords: Cobinamide, cobalamin, glucose, fructose, kinetics.

Взаимодействие кобинамида с глюкозой и фруктозой

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Изучена кинетика восстановления кобинамида (Cbi) глюкозой и фруктозой в щелочной среде. Показано, что в отличие от реакции моносахаридов с аквакобаламином (H_2OCbl), конечным продуктом которой является Cbl(II), их реакция с кобинамидом приводит к получению комплекса Co(I).

Ключевые слова: Кобинамид, кобаламин, глюкоза, фруктоза, кинетика.

Introduction

Cobalamins (Cbls, Figure 1a) are the group of organometallic compounds including cobalt ion equatorially ligated by corrin macrocycle and axially by 5,6-dimethylbenzimidazole (DMBI) and different substituents X. Cobalt ion in cobalamins can exist in +3, +2, +1 oxidation states. Cobalamin species including all three cobalt oxidation states are major participants of the following catalytic cycles: reversible $Co^{3+} \leftrightarrow Co^{1+}$ conversion in methionine synthase cycle[1] and $Co^{3+} \leftrightarrow Co^{2+}$ conversion in methylmalonyl-CoA-mutase cycle.[2]

$Co^{3+} \rightarrow Co^{2+}$ step (potential is -0.04 V *vs.* SCE, 22 °C[3]) can be easily realized *in vivo* and *in vitro*. Cobalamin(II) (Cbl(II)) can be obtained with the use of many reducing agents: ascorbic acid, borohydride,[4] formate,[5] glucose[6] and others.[4] $Co^{2+} \rightarrow Co^{1+}$ step is more complex. The mechanism of this step is a subject of detailed investigations for more then 10 years. It was known that Cbl(II) forms predominantly five-coordinated complexes[7] (an unique example of six-coordinated Cbl(II) complex is a complex with $SO_2^{\cdot-}$ anion-radical[8]), but cobalamin(I), Cbl(I), is considered to be four-coordinated.[7] The potential of five-coordinated Cbl(II)/tetra-coordinated Cbl(I) couple is -0.85 V (*vs* SCE, 22 °C)[3] that is lower than potentials of all known *in vivo* reductants.[9] The coordination state of Cbl(II) is enable modulating the potential of Co^{2+}/Co^{1+} couple: the potential of Cbl(II) with detached DMBI ($Cbl(II)_{base\text{-}off}$) reduction to Cbl(I) is increased by 0.11 V.[3] This structural feature is implemented in some enzymes since Cbl is often bound by apoenzyme in base-off conformation.[10] It is established that Cbl(II) in human ATP:cobalamin adenosyltransferase is tetra-coordinated[11] that can contribute to further increase of the potential of Cbl(II) reduction.[12] Recent dispersion corrected DFT calculations showed the ability of Cbl(I) to form a weak bond with hydrogen atom of different molecules.[13]

The calculated potential of five-coordinated Cbl(II)/five-coordinated Cbl(I) couple has a value that is within the range of *in vivo* reductants potentials.[14]

Cobinamide (Cbi, Figure 1b) is a cobalamin derivative lacking DMBI moiety. Recently this compound has attracted researchers' interests due to the high efficacy of its derivatives as cyanide antidotes (sulfitocobinamide)[15-17] and cyanide chemosensors (monocyanocobinamide).[18-20] Cbi application is also of interest in the study of both $Co^{3+} \rightarrow Co^{2+}$ and $Co^{2+} \rightarrow Co^{1+}$ reduction mechanisms to establish the role of lower axial base in this process.

Earlier we have shown that monosaccharides are efficient reducing agents of cobalt tetrasulfophthalocyanine (CoTSPc),[21] and also demonstrated the ability of glucose to reduce Cbl(III) to Cbl(II) at strong alkaline conditions.[6] In this work the investigation of reduction kinetics of Cbi(III) to Cbi(II) and of Cbi(II) to Cbi(I) by glucose and fructose was performed in alkaline media, the mechanisms of the reactions were suggested and the influence of complex structure on the possibility of Co(I) formation was established.

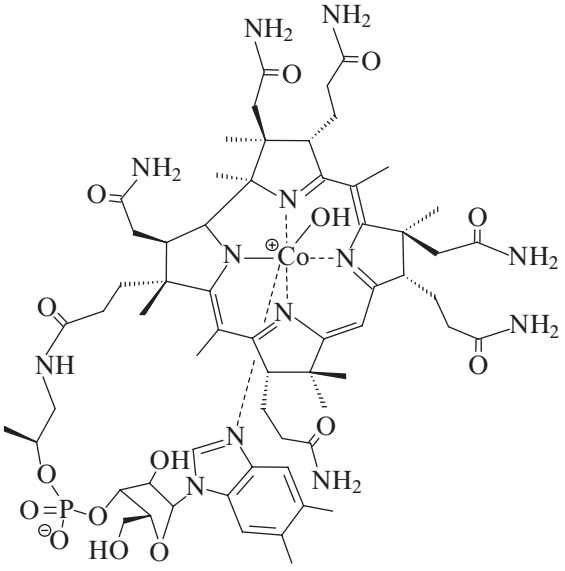

Figure 1. Structural formulas of hydroxocobalamin (a) and dihydroxocobinamide (b).

Experimental

*Materials***.** Aquahydroxocobinamide was prepared by acid hydrolysis of hydroxocobalamin and purified by reported procedure.[22] Purity of product was controlled by UV-vis spectrometry[23] and chromatography methods.

Hydroxocobalamin hydrochloride (HOCbl, ≥98 %), *D*-(+)-glucose, *D*-(–)-fructose were purchased in Sigma-Aldrich and used as received. Other chemicals used throughout this work were of analytical reagent grade. Doubly distillated water was used in all measurements. Oxygen-free argon was used to deoxygenate solutions.

Kinetic measurements. Kinetics was studied on a thermostated UV-vis spectrophotometer Cary 50 at anaerobic conditions at the excess of monosaccharides. Kinetics for Cbi(III) → Cbi(II) and Cbi(II) → Cbi(I) reduction steps was monitored by change in absorbance at 530 and 388 nm, respectively. Experimental data were analyzed using Origin 7.5 software.

Results and Discussion

Reaction of Cbi(III) with Glucose and Fructose

Cbi(III) reacts with glucose (Glc) at p*H* > 11, that results in a solution color change from red to yellow. The increase of absorption at 266, 314, 469 nm is observed at p*H* up to 12.4 (the UV-vis spectrum of reaction product is shown in Figure 2, spectrum *2*) that corresponds to the UV-vis spectrum of Cbi(II).[24] The similar spectrum may be obtained using the reduction by ascorbic acid or sodium formate. Cbi(III) reacts with fructose (Frc) at p*H* > 10.4 with the Cbi(II) formation at p*H* range from 10.4 to 12 (Figure 2, spectrum *2*).

At higher p*H* values there is the occurrence of a consecutive process in addition to Cbi(III) → Cbi(II) stage. The solution color changes from yellow to gray and maxima in UV-vis spectrum appear at 300, 388, 458, 548, 680 nm (the UV-vis spectrum of reaction product is shown in Figure 2, spectrum *3*). The UV-vis spectrum is identical to Cbi(I).[12]

The mixing of Cbi(III) with fructose at p*H* 10.6 results in fast changes in UV-vis spectrum preceding a consecutive

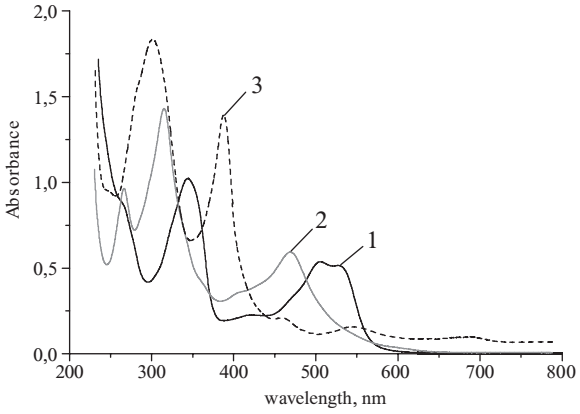

Figure 2. UV-vis spectra of dihydroxocobinamide(III) (spectrum 1), cobinamide(II) (spectrum 2), cobinamide(I) (spectrum 3). [Cbi] = $5 \cdot 10^{-5}$ M; 25 °C.

significantly slower Cbi(II) formation: absorption increase in the range between 360-490 nm and its decrease between 490-570 nm are observed (Figure 3). The spectral changes are absent in neutral media and significantly less pronounced in the case of glucose. Probably, this stage corresponds to the coordination of deprotonated fructose (*vide infra*). The determination of the equilibrium constant of Frc^- binding by aquahydroxocobinamide was impossible due to the insufficient curvature of absorbance dependence on [Frc].

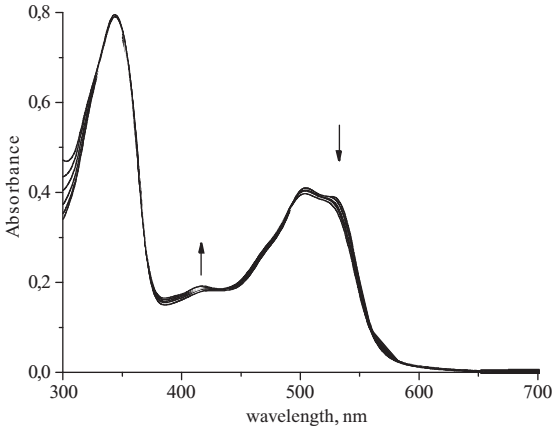

Figure 3. UV-vis spectra of cobinamide(III) in 0-0.5 M fructose solutions (arrows show increase in fructose concentration). [Cbi] = $5\cdot10^{-5}$ M; p*H* 10.6; 0.2 M carbonate buffer; 25 °C.

Cbi(III) reacts with Glc at p*H* < 12 with very low rates, therefore these reactions were studied at p*H* ≥ 12. Kinetic traces for the reaction of Cbi(III) with Glc at p*H* ≤ 12.4 (Figure 4, curve *1*) fit well to a first-order rate equation that indicates the first order of reaction with respect to cobinamide. Linear absorption decay at 530 nm *versus* time (Figure 4, curve *2*) was observed at more alkaline conditions (p*H* > 12.4). The kinetics of the reaction of Cbi(III) with Frc at p*H* 11.4–12.3 is described by a first-order reaction equation (the shape of kinetic curve is identical to that of the reaction of Cbi(III) with Glc shown in Figure 4, curve *1*). At p*H* = 12.3-13.0 shape of kinetic traces was linear, but at p*H* > 13 kinetic curve had an inflection (not shown in this work).

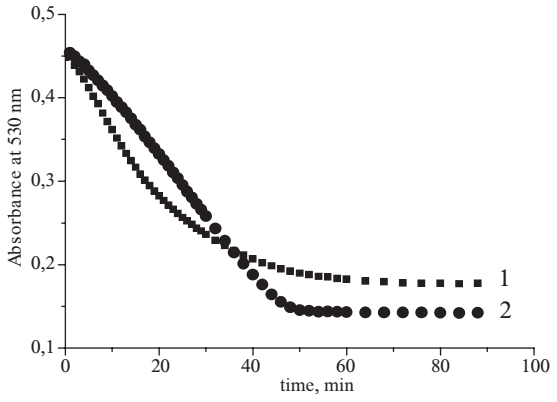

Figure 4. Plot of absorbance at 530 nm *vs.* time for the reaction of Cbi(III) with glucose at p*H* 12.4 (1) and 12.8 (2). [Cbi] = $4.4\cdot10^{-5}$ M; [Glc] = 0.1 M; 25°C.

We performed experiments with different initial Cbi(III) concentrations and constant other conditions ([Glc] = 0.1 M; p*H* 12.7; 25 °C) for the exact establishment of reaction order with respect to Cbi(III) under strongly alkaline conditions. We have found that reaction rates in these experiments were not the same and linearly depended on initial Cbi(III) concentration. It is likely that kinetic curves under these conditions include two parts: initial part of exponential trace, slope of that is clearly dependent on [Cbi(III)]; following part "linearized" by means of rate increase by active species derived from monosaccharides and accumulating in system during the process. For this reason reaction rates in strongly alkaline media were calculated using initial slopes of kinetic curves.

Note that in strongly alkaline media the self-reduction of Co^{3+}-corrinoids to Co^{2+}-species occurs.[6,25] We have found that the observed rate constant of dihydroxocobinamide self-reduction linearly depends on $[OH^-]$. The rate constant of this process obtained as a result of observed rate constant dividing by $[OH^-]$ has a low value ($k_{s.-red.}$=(1.46 ± 0.03)·10^{-4} $M^{-1}s^{-1}$, 25 °C), thus this process practically does not influence on the kinetics of Cbi(III) reduction by monosaccharides.

We found that the rate of Cbi(III) reduction by glucose linearly increases with $[OH^-]$ growth (Figure 5). This indicates the first reaction order with respect to OH^-.

The dependence of k_{obs} on $[OH^-]$ for the reaction of Cbi(III) with Frc was obtained at p*H* ≤ 12. This dependence

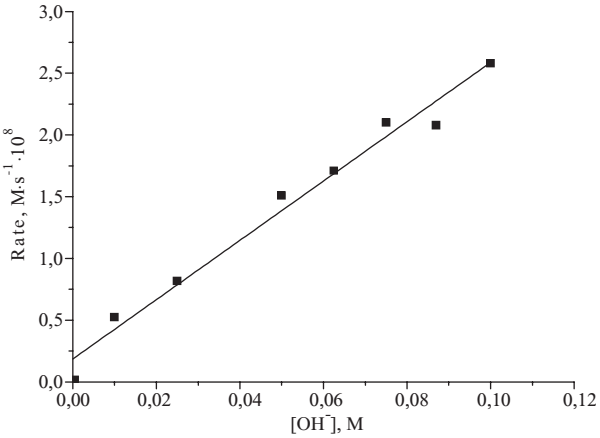

Figure 5. Plot of the rate of Cbi(III) reduction by glucose *vs.* $[OH^-]$. [Cbi] = $5\cdot10^{-5}$ M; [Glc] = 0.1 M; 25°C.

is shown in Figure 6. It is nonlinear, but can be linearized in coordinates $k_{obs}\times1/(\text{fraction(Cbi(III)}(OH^-)(H_2O))$ *versus* $[OH^-]$. This suggests the importance of the protonation of inert dihydroxocomplex in the course of first reduction step (*vide infra*).

The dependence of reaction rate on glucose concentration was studied at p*H* 13 (Figure 7). It is linear in the range of glucose concentrations between 0-0.06 M. There is also a positive intercept, probably, indicating the presence of either parallel or reverse reaction.

The dependence of k_{obs} on [Frc] was obtained at p*H* 11.4 (Figure 8). It can be seen that k_{obs} linearly depends on [Frc] that indicates first reaction order with respect to reducing agent.

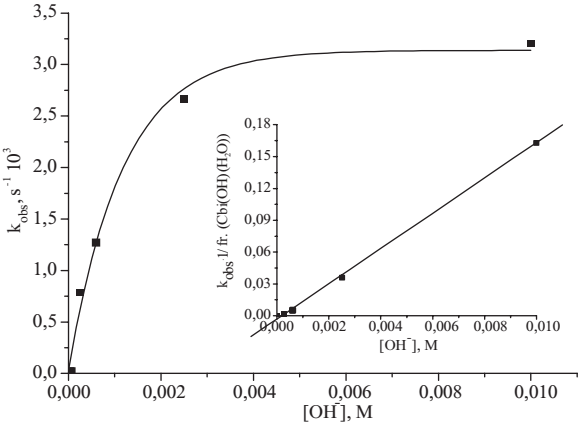

Figure 6. Plot of observed rate constant *vs.* [OH$^-$] for the reduction of Cbi(III) by fructose. Inset: Plot of k_{obs}/fraction(Cbi(III)(OH$^-$)(H_2O)) *vs.* [OH$^-$]. [Cbi] = 5·10^{-5} M; [Frc] = 0.1 M; 25 °C.

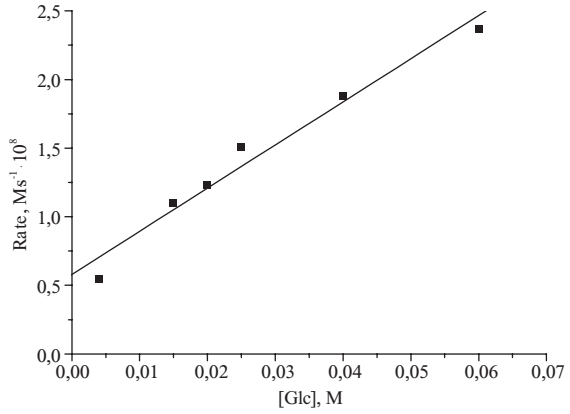

Figure 7. Plot of the reduction rate of Cbi(III) by glucose *vs.* [Glc]. [Cbi] = 5·10^{-5} M; p*H* 13; 25°C.

In alkaline media equilibrium between aquahydroxocobinamide(III) and dihydroxocobinamide (Reaction 1) (pK_a = 10.3 at 25°C) occurs.[23] Dihydroxo-form possesses a low reactivity, consequently, aquahydroxo-form produced as the result of the fast protonation of dihydroxo-form is to be an active species during the Cbi(III) reduction by monosaccharides.

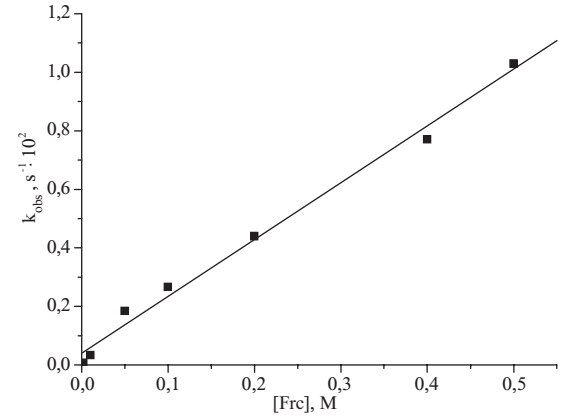

Figure 8. Plot of observed rate constant *vs.* [Frc] for the reduction of Cbi(III) by fructose. [Cbi] = 5·10^{-5} M; p*H* 11.4; 25°C.

$$H_2O–Cbi(III)–OH^- \leftrightarrow {}^-HO–Cbi(III)–OH^- + H^+ \quad (1)$$

It is well-known that neutral species of glucose and fructose (GlcH, FrcH) undergo deprotonation under alkaline conditions and are transformed to ionized enol species (Glc$^-$, Frc$^-$) (Reaction 2) becoming stronger reductants. pK_a values for glucose and fructose ionization are 12.28 and 12.03 at 25°C.[26] Rate constants for forward Reaction 2 are 1.7·10^{-3} and 1.4·10^{-2} M^{-1}s^{-1} at 25 °C for glucose and fructose, respectively.[27] Since no evidence of the reaction was observed under both acidic and neutral conditions, it may be concluded that ionized species react with Cbi(III) (Reaction 3).

$$GlcH(or\ FrcH) + OH^- \leftrightarrow Glc^-(or\ Frc^-) + H_2O \quad (2)$$

On the basis of experimental data we can conclude that the reduction of Cbi(III) by fructose at p$H \leq 12$ proceeds by the other route. Apparently, under these conditions the reaction proceeds *via* the fast formation of the complex between Cbi(III) and Frc$^-$ (shown in Figure 3) and a subsequent electron transfer from Frc$^-$ to Co^{3+} (Reactions 3, 4).

$$H_2O–Cbi(III)–OH^- + Frc^- \leftrightarrow {}^-Frc–Cbi(III)–OH^- + H_2O \quad (3)$$

$$^-Frc–Cbi(III)–OH^- + H_2O \rightarrow Cbi(II)–H_2O + Frc^{\bullet} + OH^- \quad (4)$$

Earlier we have studied the reduction of Cbl(III) by glucose in alkaline media.[6] That reaction had the first order with respect to glucose and was significantly slower. The facts sustain the necessity of aqua-form formation for a further reaction with reducing agent. Since pK_a for aquacobalamin deprotonation is 7.8 at 25°C[28] (that is significantly lower than pK_a for aquahydroxocobinamide deprotonation), the concentration of aquacobalamin in alkaline media is significantly lower than the concentration of aquahydroxocobinamide. The presence of tightly bound DMBI in cobalamin (that cannot be substituted by monosaccharide molecule) is also a factor decreasing reaction rate by diminishing the number of potentially available reaction sites.

Reaction of Cbi(II) with Glucose and Fructose

The typical kinetic trace of Cbi(I) formation using both glucose and fructose as reducing agents fits nicely to a first-order exponential equation (not shown in this paper) indicating the first reaction order with respect to cobinamide.

Dependences of observed rate constant for the reduction of Cbi(II) to Cbi(I) by glucose and fructose on OH$^-$ concentration were obtained (Figure 9). These dependences are nonlinear, but are linearized in coordinates k_{obs} *versus* [OH$^-$]^2. This is evidence of the second order of reaction with respect to OH$^-$. Therefore, two hydroxide ions take part in the reduction of Cbi(II) by monosaccharides.

There are the dependences of observed rate constants for the reduction of Cbi(II) to Cbi(I) by monosaccharides on both glucose and fructose concentrations in Figure 10. These dependences are nonlinear, but could be linearized in $1/k_{obs}$ *vs.* 1/[Glc(Frc)] coordinates. This fact is the consequence of the reversible formation of intermediates between Cbi(II)

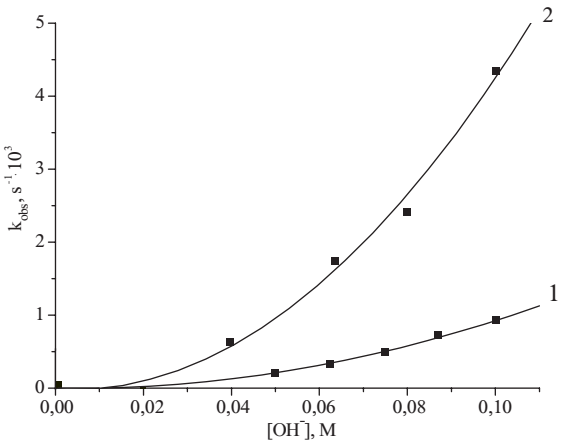

Figure 9. Plot of observed rate constant for the reduction of Cbi(II) by glucose (1) and fructose (2) *vs.* $[OH^-]$. [Cbi] = $5\cdot10^{-5}$ M; [Glc] = [Frc] = 0.1 M; 25°C.

and monosaccharides and its further decomposition. Special experiments showed no inhibition of Cbi(II) → Cbi(I) stage by reaction products. We also found that there is no reaction occurrence between Cbi(II) and both monosaccharides in acidic media. Probably, the reduction of Cbi(II) proceeds *via* the formation of enols.

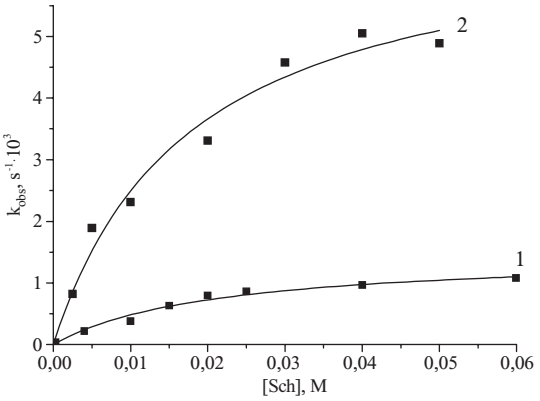

Figure 10. Plot of observed rate constants of Cbi(II) reduction by glucose (1) and fructose (2) *vs.* monosaccharides (Sch) concentration. [Cbi] = $5\cdot10^{-5}$ M; p*H* 13; 25°C.

As it was noted above, the reduction of Cbi(II) by monosaccharides requires two hydroxide-ions. Probably, the first OH^- ion reacts with neutral monosaccharide molecules that result in enol formation (Reaction 2). Presumably enol takes part in the formation of intermediate with Cbi(II) (Reaction 5), which reacts with the second OH^- ion leading to the formation of Cbi(I) and products of monosaccharide oxidation (Reaction 6).

$$\text{Cbi(II)–H}_2\text{O} + \text{Glc}^-(\text{or Frc}^-) \leftrightarrow \leftrightarrow \text{Cbi(II)–Glc}^-(\text{or Frc}^-) + \text{H}_2\text{O} \quad (5)$$

$$\text{Cbi(II)–Glc}^-(\text{or Frc}^-) + \text{OH}^- \rightarrow \text{Cbi(I)} + \text{products} \quad (6)$$

The experimental data show that both Cbi(I) and Cbi(II) formation in the case of fructose is faster than the reaction with glucose. The same observation was previously pointed out during Co(II)TSPc reduction by these substances.[21] It can be explained by the fact that enol formation depends directly on the ratio of open and cyclic monosaccharides species concentrations in solution. The concentration of open form in fructose solution is higher than that in glucose solution,[29,30] that probably explains the greater reactivity of the first monose. Values of pK_a and rate constants of ionized enols formation quantitatively explain that Frc^- is produced faster and at lower p*H* values than Glc^- (*vide supra*).

It should be noted that these monosaccharides can not reduce cobalamin(II). This observation supports the necessity of reductant coordination on Co^{2+} for the further reduction stage since relatively tightly bound DMBI is present in Cbl(II) molecule[4] which probably can not be substituted by monosaccharide molecule during the process. Relatively weakly bound water molecule is present in Cbi(II),[31] that is likely to be easily substituted during the reaction of metallocomplex with monosaccharide. The coordination of reducing agent on Cbi(II) implies that the formation of Cbi(I) in this case is a result of inner sphere electron transfer.

Conclusions

This work demonstrates the ability of both glucose and fructose to reduce cobinamide(III) to cobinamide(II) and cobinamide(II) to cobinamide(I) in alkaline media. It was demonstrated that the formation of ionized enol is to be a key factor for the Cbi(III) to Cbi(II) reduction. The route of Cbi(III) reduction by fructose under the moderately alkaline condition involving the formation of the intermediate complex with ionized fructose enol form was proposed. Both the necessity of monosaccharide derivatives coordination on Cbi(II) for the further metallocomplex reduction and the involvement of two OH^- ions in this process were demonstrated.

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