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ISONITRILES (ISOCYANIDES) AS MODEL COMPOUNDS IN THE INVESTIGATION OF THE REACTION MECHANISM AND KINETICS OF THE LIGANDS WITH HEME IRON IN HEMOGLOBINS, MYOGLOBINS, AND CYTOCHROMES

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Abstract. A brief historical review of the investigations of the reaction mechanism and kinetics in the reactions of isonitriles with heme iron in hemoglobins, myoglobins, and cytochromes is given. It is shown how from the hypothesis of Pauling with the throughgoing investigations by others a real "pocket" model to describe the forming of the binding of ligands with ferro-heme was reached. An abstract of toxicity of isonitriles is also given.

Key words: isonitrile, isocyanide, heme, hemoglobin, myoglobin, cytochrome.

Abbreviations: Hb = hemoglobin; Mb = myoglobin; Cyt = cytochrome; MeNC = methyl isonitrile; EtNC = ethyl isonitrile; PrNC = propyl isonitrile; iPrNC = isopropyl isonitrile; nBuNC = *n*-butyl isonitrile; t-BuNC = *tert*-butyl isonitrile; PNC = pentyl isonitrile; nHNC = *n*-hexyl isonitrile; cHNC = cyclohexyl isonitrile; BzNC = benzyl isonitrile; TSM–NC = *p*-toluenesulphonyl methyl isonitrile.

The isonitriles *per se* are of growing interest. So in the *Chemical Abstracts* (C.A.) vol. 75 (1971) under the key word "isocyanide" there are 12 abstracts, but in vol. 126 (1997) only under the key words "acetic acid", "benzene", "ethane", "methane", and "propane" there are 144 abstracts of investigations that include isonitriles.

The isonitriles as starting compounds (Passerini and Ugi reactions) in the organic synthesis give many possibilities for the synthesis of biologically active substances [1, 2]; on the other hand, the isonitriles have been used as model compounds in the investigations of the reaction mechanisms and kinetics of the

ligands with heme iron in hemoglobins (Hbs), myoglobins (Mbs), and cytochromes (Cyts). For the chemists working with the isonitriles it is also essential to know how the isonitriles may react physiologically. A brief review on the literature in this area is given below. Historically Warburg, Negelein & Christian [3] were the first to exhibit that methyl isonitrile (MeNC) combines with horse Hb with an affinity of approximately one fourtieth that of oxygen. Russel and Pauling found that ethyl isonitrile (EtNC) combines with ferro-Hb and that the resulting compound EtNC–ferro-Hb is diamagnetic, the bonds between the iron atom and its surrounding atoms being essentially covalent [4], as in oxyhemoglobin.

St. George & Pauling [5] showed that there is a big difference in the combining power of EtNC, isopropyl isonitrile (iPrNC), and *tert*-butyl isonitrile (t-BuNC) with Hb, which indicates the steric hindrance effect in the Hb, but these three isonitriles have almost the same combination constants with ferroheme itself. It was supposed that the shape of the protein layers in the neighbourhood of the alkyl group of the isonitrile may be such that the isopropyl group can fit into the protein with narrowly greater dilation than is caused by the ethyl group, whereas the tertiary butyl group might exert a much greater steric effect. This situation was described as a "hollow" which the larger *tert*-butyl group is not able to fit. The "hollow" is the same "pocket" used later by Gibson, Olson, and others in their models.

Lein & Pauling [6] exhibited that horse Mb like Hb combines approximately 200 times more strongly with EtNC than with t-BuNC. This great dependence of the value of the combining constant on the nature of the alkyl group has a reasonable explanation in steric hindrance, and it is concluded that in Mb, as in Hb, the heme group is not attached to a surface of the globin molecule, but is buried with it.

Pauling with coworkers [5, 6] showed the important steric effects from the size of hydrocarbon radical of isonitriles in the reaction with Hb and Mb. This effect was investigated later in more detail by other scientists.

Keilin [7] shows that MeNC is attached to the heme iron through its carbon atom as well as cyanide ion. MeNC, like carbon monoxide, combines only with heme and Hb and does not react with the ferric compounds of hematin and methemoglobin, which both form characteristic compounds with cyanide ion.

Okazaki & Tsushima [8] investigated the reaction of EtNC with horse and dog Hb, and they showed that the dissociation constant of the Hb–EtNC compound is dependent on pH.

Ainsworth, Gibson & Roughton [9] showed that the initial rate of the combinations of isonitriles from methyl to *tert*-butyl with Hb and with Mb decreases with increasing chain length approximately 500-fold with Hb and approximately 40-fold with Mb. At the same time the rate of dissociation of MeNC from Hb is approximately 50 times greater than that of all the higher homologues.

Antonini published a review [10] about the reaction of ligands (e.g. alkyl isonitriles) with Hb, about their reaction kinetics, structure of compounds, and reaction mechanism. The detailed set of crystallographic data concerning the structure of Mb and Hb presented by Perutz and coworkers [11] also permit better understanding of the reactions of ligands with Hb and Mb.

With the work of Brunori & Schuster [12] the detailed investigation of ligand binding to Hb with different methods began. In addition, they investigated the kinetics of the binding of EtNC to human Hb by the temperature jump relaxation method and exhibited that the relaxation spectrum of EtNC binding to Hb is qualitatively similar to that observed in the oxygen reaction. They also remark that the complete reaction mechanism for Hb is not yet known.

Gibson developed a technique for kinetic measurements using the apparatus of the stopped flow method [13]. This method has been used later also in the investigations of reaction kinetics of isonitriles with Hb.

Olson & Gibson [14] showed that the binding of *n*-butyl isonitrile (nBuNC) to human Hb requires the consideration of a minimum of two components and these represent the α and β subunits of Hb [10] and that the organic phosphates exert an unequal influence on the two types of heme-binding sites in human Hb. In the extreme case of inositol hexaphosphate–Hb only the β chains bind ligand up to 20% saturation of the total heme sites with nBuNC. They suppose that ten intermediates are required for an adequate description of the reaction of nBuNC with stripped Hb and if one now adds phosphates to such a scheme, the number of intermediates is increased by a factor or two.

The association–dissociation phenomena in Hb were examined also by Anderson, Antonini, Brunori & Wyman [15]. They reached a conclusion that the equilibrium of human Hb with EtNC resembles in its general properties that of oxygen or carbon monoxide. A detailed picture of the equilibrium between human Hb and EtNC was given, which is evidence that αβ dimers of Hb obtained on the dissociation of tetrameric Hb have a high degree of co-operativity, and suggests that they are responsible for a large fraction of the total functional interactions present in the tetrameric molecule of Hb. They also state that the interpretation of the functional behaviour of Hb is far from being settled.

Olson & Gibson [16] published detailed investigations of the reactions of Hb with isonitriles. They showed that the α and β chains within the human Hb tetramer possess different reactivities towards nBuNC. The β chain association and dissociation rate constants were both significantly greater than the corresponding α chain constants. The equilibrium constants for both chains were roughly equivalent. On the other hand, Talbot, Brunori and coworkers [17, 18] have also begun an extensive investigation of the reaction of hemoproteins with alkyl isonitriles. In the first paper of this series [17] they also show that the reactions of alkyl isocyanides with the isolated α and β chains of human Hb resemble in their general properties those with other ligands, such as O_2 or CO, and in reactions of each hemoprotein with larger isonitriles the equilibrium and

kinetic constants decrease monotonically with the increase in the ligand size, which may indicate steric effects. The authors state that their paper from 1971 [17] is the first in which the equilibria and kinetics of the reaction of α and β chains of human Hb with alkyl isonitriles has been reported. An explanation of the steric effect is presented by stating that the aliphatic side-chain of the ligand tends to be packed toward the interior of the molecule in a nonpolar environment. Therefore, binding would be favoured by the transfer of the hydrocarbon chain from water to the nonpolar environment and hindered by steric effects which become more and more serious as the size of the ligand increases [18].

In the determination of the velocity constants of the reaction of isonitriles with Hb (carp and *Chironomus*) Ruckpaul and coworkers [19] widened the investigation area of Hbs.

Olson & Gibson [20] showed in the following investigation that the functional heterogeneity observed for the reactions of BuNC with the α and β chains of human Hb at pH 7 is absent at pH 9.1, which exhibits that the chains possess similar ligand-binding in alkaline conditions.

The photodissociation of carbon monoxide and alkyl isocyanides from Hb was indicated already by Antonini & Brunori [21]. Irwin & Stynes [22] investigated the same phenomenon at first with benzyl isonitrile (BzNC) on heme models and showed that the quantum efficiency for photodissociation of CO and BzNC in these systems is similar to that reported for Hb.

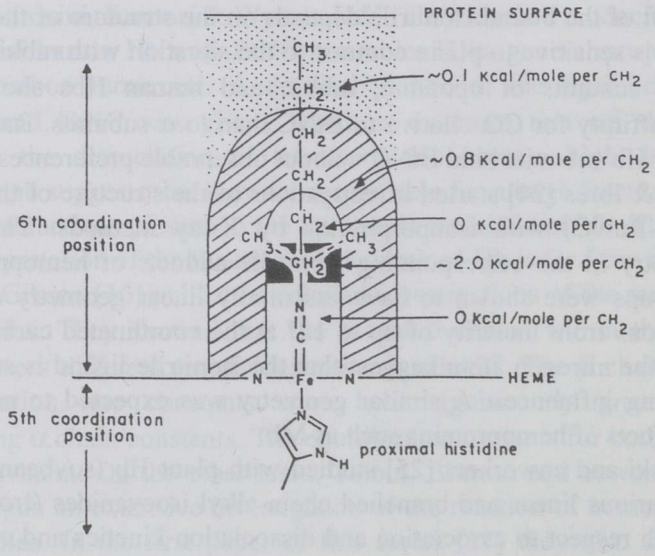
Interactions between EtNC and iPrNC and various Hbs and Mbs by ^{13}C NMR have been studied by Dill and coworkers [23]. Their results indicate that the chemical shift of the bound isonitrile depends on the structure of the Hb subunit or Mb and it is sensitive to pH in contrast to the situation with rabbit and human Hbs. The β subunits of opossum, rabbit, and human Hbs show a greater preferential affinity for CO relative to EtNC than to α subunits. Rabbit, human, and opossum Hb subunits bind EtNC without observable preferences.

Jameson & Ibres [24] started investigations of the structure of the binding of isonitriles (t-BuNC) with ironporphyrins by X-ray methods. They expected linear geometry in the corresponding isonitrile adducts of hemoproteins. Both Fe–CNR groups were shown to have essentially linear geometry although the large distortions from linearity of up to 12° at the coordinated carbon atom and up to 21° at the nitrogen atom suggest that the isonitrile ligand is susceptible to crystal packing influences. A similar geometry was expected to pertain to the isonitrile adducts of hemoproteins such as Mb.

Stetzkowski and coworkers [25] studied with plant Hb (soybean leg-Hb) the binding of various linear and branched chain alkyl isocyanides (from methyl to *n*-heptyl) with respect to association and dissociation kinetics and compared the results with those obtained in parallel on sperm whale and horse heart Mbs. They concluded that the isocyanide binding to leg-Hb is with a greater flexibility of the heme pocket than to the Mbs.

Traylor and coworkers studied the binding of nBuNC, t-BuNC, and *p*-toluenesulphonyl methyl isonitriles (TSM–NC) to chelated protoheme [26, 27]. The affinities of the isonitriles (RCN) correlate with the electron-withdrawing properties of R, the electron-withdrawing tosylmethyl group enhancing the π-acceptor properties of the isonitrile and thus binding more strongly. Comparison with isonitrile binding to proteins shows ~ 10^4 times poorer binding of nBuNC to Mb compared to protoheme monomethyl ester mono-3-imidasolylpropylamide and over 10^5 times for t-BuNC or TSM–NC. The large differences are due to distal steric effects present in the restricted pocket of Mb, which are not present in chelated protoheme.

Reisberg & Olson [28] studied reactions of human Hb with 11 alkyl isonitriles (methyl, ethyl, propyl, iso-propyl, *n*-butyl, isobutyl, (+)- and (–)-*sec*-butyl, *tert*-butyl, *n*-pentyl, *n*-hexyl), and cyclohexyl isonitrile (cHNC) and BzNC. They showed that there appears to be no increase in steric hindrance in going from MeNC to EtNC. For the next two isonitriles, propyl isonitrile (PrNC) and nBuNC, the situation is quite different. In their case the additional carbon units appear to reside in an area of high steric hindrance (~ 1.5 kcal/mole/CH_2). The markedly lower affinity of Hb for cHNC when compared to the *n*-hexyl isonitrile (nHNC) is due to the smaller surface area and, therefore unbound potential of the cyclocompound. Binding properties of (+)- and (–)-*sec*-butyl isonitriles allowed the authors to suggest that there are no highly stereospecific constraints at the ligand binding site in Hb.



Free energy map of unfavourable steric interactions on the distal side of the heme group in mammalian hemoglobins and myoglobins [28, 32].

The potential diagram of the ligand binding site in Hb (Fig.) is very interesting. In this diagram the different regions indicate the increases in bound ligand potential due to adding a carbon unit to different parts of the alkyl side chain. The free energy results show a cavity at the level of the terminal carbon group of EtNC (open area) surrounded by a region of large steric interaction (hatched area) and an outer area of smaller interaction (dotted region). Hb exhibits high affinity for BzNC, particularly when compared with the cHNC, which has a similar surface area. It is possible that this is due to a favourable π-electron cloud interaction between the ligand side chain and aromatic amino acid residues (e.g. distal histidine and phenylalanines) at the binding site.

Reisberg & Olson [29, 30] also exhibited by the reaction of 13 isonitriles to the isolated α and β subunits of human Hb that the first step in the binding process appears to be a rapid dissolution of the ligand into certain hydrofobic regions of the protein molecule. The second step involves ligand diffusion through a more rigid structure near the final binding site, and the last step is bond formation between the isonitrile carbon atom and the iron atom of the heme group.

Brunori with coworkers [31] investigated the reactions of the trout Hb with isonitriles and the pH-dependence of the two major components of trout hemolysates.

Mims et al. [32] investigated the O_2, CO, and alkyl isonitrile (methyl, ethyl, *n*-propyl, *n*-butyl, *n*-amyl, *n*-hexyl) binding properties of a variety of vertebrate and invertebrate heme proteins comparing these in detail to those of protoheme mono-3-(1-imidasoyl)-propylamide monomethyl ester in aqueous suspensions of soap micelles. The proteins examined include different Cyts, Hbs, and Mbs, and the α and β subunits of human Hb. In the case of the proteins, a marked decrease in the association rate constant is observed in going from CO to MeNC. The slow rates of isonitrile to Cyt *c* oxidase could be easily explained considering that the total length of the Fe═C═N—CH_3 complex in a linear geometry is about 5 Å and so the steric hindrance by the Cu atom (Cyt!) or one of its ligands causes the extremely slow rates. Also the polar or ionic character of the active site in Cyt *c* peroxidase appears to inhibit the alkyl isonitrile from reaching the heme iron atom.

The binding of mammalian Hbs and Mbs with ligands when compared to CO and alkyl isonitriles MeNC binding is sterically hindered in the active site from the placement of the third ligand atom, by EtNC the effect is the same. Going from EtNC to nBuNC the increase is linear (~0.8 kcal/mole per CH_2). The surrounding area occupied by EtNC is a larger region of restrictive protein structure into which the atoms of PrNC, iPrNC, nBuNC, and t-BuNC extend. Still farther from the heme is a more fluid region into which the terminal carbon atoms on pentyl isonitrile (PNC) and nHNC protrude.

Olson and coworkers [29, 32] showed that ligand binding involves a minimum of three distinct processes and can be considered in terms of the following equation:

$$P + X \underset{}{\overset{K_s}{\rightleftharpoons}} C \overset{K_p}{\rightleftharpoons} B \overset{K_b}{\rightleftharpoons} PX$$

1. Solvent or hydrophobic effect

2. Cage or diffusion effects
3. Protein distal effects

4. Intrinsic bond strength
5. Protein promixal effects

P, protein molecule; X, ligand molecules.

State C represents a ligand dissolved in outer hydrocarbon regions of the protein molecule; state B is a ligand molecule in the sixth coordination position with the correct orientation, but not yet bound to the heme iron atom; and PX is the final iron–ligand complex.

Steric interactions between Hb and Mb bound ligand molecules and protein (the valine E11 methyl groups) were examined directly with high resolution NMR techniques by Mims et al. [33]. They suggest a linear geometry for the Fe═C═N—C bonds in the β chains and a bent geometry for the α chains of Hb. The bent geometry in the α subunit appears to be dictated by the position of the valine E11 methyl group, which is located further up from the heme plane, but closer to the heme centre than the corresponding position of the β subunit residue.

For kinetic description the nanosecond recombination time courses were measured [34] by photolysing O_2, NO, CO, MeNC, EtNC, PrNC, nBuNC, and t-BuNC complexes of sperm whale Mb. The reaction is described as follows:

$$MbX \rightleftharpoons B \rightleftharpoons C \rightleftharpoons Mb + X$$

B, geminate state in which the ligand is present in the distal pocket but not covalently bound to the iron atom;
C, ligand is still embedded in the protein but further away from the heme group.

Three major conclusions were derived from these analyses.

1. The overall guantum yield, Q, of the ligand complex is determined primarily by the competition between the rate of iron-ligand bond formation from the initial photoproduct, $k_{B\rightarrow MbX}$, and the rate of migration away from state B, $k_{B\rightarrow C}$. For example, $k_{B\rightarrow C} \simeq 30 - 100\ \mu s^{-1}$ for all three gaseous ligands, whereas both Q and $k_{B\rightarrow Mb\,X}$ vary over 3 orders of magnitude (i.e. NO, $Q = 0.001$, $k_{B\rightarrow MbX} \simeq 16\,000\ \mu s^{-1}$; O_2, $Q = 0.1$, $k_{B\rightarrow MbX} \simeq 500\ \mu s^{-1}$; CO, $Q = 1.0$, $k_{B\rightarrow Mb\,X} \simeq 2\ \mu s^{-1}$).

2. For NO, O_2, and the isonitriles, the rate-limiting step in the overall association reaction starting from ligand in solution is the formation of state B. The rate constant for this process varies from $2 \times 10^7\ M^{-1}\ s^{-1}$ for the gaseous

ligands to $0.02 - 1.4 \times 10^5\ M^{-1}\ s^{-1}$ for the isonitriles. In contrast, the *B* to Mb*X* transition is limiting for CO binding.

3. For all the ligands except CO, the overall rate of dissociation is limited significantly both by the rate of thermal bond disruption, $k_{Mb\ X \to B}$, and the competition between geminate recombination and migration away from the distal pocket (i.e. $k_{B \to C} / (k_{B \to Mb\ X} + k_{B \to C})$). In the case of CO, the rate of bond disruption is equal to the observed dissociation rate constant.

The optical absorption and ligand binding properties of newly reconstituted sperm whale Mb were examined systematically at pH 8.0, 20°C by Olson and coworkers [35]. The equilibrium and kinetic parameters for O_2 and CO binding to newly reconstituted Mb were observed to be identical to those of the native protein. The orientation of the heme group has no effect on the physiological properties of Mb. Significant kinetic heterogeneity was observed only for long-chain isonitriles.

Tsubaki et al. [36] investigated the interactions of cholesterol analogues and inhibitors with the heme moiety of Cyt P-450_{SCC}. They applied resonance Raman spectroscopy. The Raman spectra of ferric cytochrome P-450_{SCC} complexed with inhibitors such as cyanide, phenyl isonitrile, aminogluthenhimide, and metyrapone were shown to be characteristic of low-spin state and to be very similar.

Wood et al. [37] have investigated the binding of aromatic isonitriles to sheep Hb and horse heart Mb. They showed that the disubstituted ligands 2,6-dimethylphenyl isonitrile were bound to the horse heart Mb with affinities ranging 500–5000-fold greater than those of ethylisonitrile, which was the tightest binding isonitrile ligand for Mb reported. An explanation for the unexpectedly high affinity is offered in terms of the electronic structure of aromatic isonitrile.

By investigating the absorption spectra of Cyt P-450 of liver and olfactory epithelium microsomes combined with isonitriles the existence of two forms of enzyme–ligand complex was shown: (1) a form with the ligand combined only with the heme Fe and (2) a form in which the ligand binds to both the heme Fe and the lipophilic substrate-binding site [38]. Aromatic isonitriles are better π-acceptors than aliphatic isonitriles and have a higher affinity for the heme than the lipophilic site.

Rohlfs, Olson & Gibson [39] showed that the rate limiting step for CO, O_2, NO, and isonitrile (methyl, ethyl, *n*-propyl, and *n*-butyl) binding to five proteins (soybean leg Hb, monomeric component π of *Glycera dibranchieta* Hb, sperm whale Mb, α and β subunits of human Hb) is the ligand migration up to the initial geminate state, and the rate of this process determines the overall bimolecular association rate constant of these ligands. In contrast, the iron-ligand bond formation limits the overall bimolecular rate for CO binding.

It was shown by Ozaki and coworkers [40] that 2,6-dimethylphenyl isonitrile is a ligand of ferrohemoglobin. The results are consistent with the binding of the

nitrogen atom of the nitroso group of nitrosobenzene and of the carbon atom of the isonitrile group of phenyl isonitrile to ferroheme.

With the use of colliding-pulse laser the recombination kinetics of ligands to Mb was studied by Jongeward et al. [41]. On the picosecond time scale all ligands investigated except CO showed a substantial amount of rebinding of the isonitriles to iron, which is surprising as they are isoelectronic with CO and might be expected to show the same or similar recombination kinetics: the difference may be due to the greater Lewis basicity of the isonitriles. All ligands, again except CO and sometimes NO, also undergo recombination from geminate pairs. This process is a thousand times slower and occurs on the nanosecond time scale. The geminate recombination of oxygen and several isonitriles with whale Mb is at least a two-intermediate process and can be modeled with a four-state model.

Differentiation between ligands occurs as the ligand enters the protein and not at the iron binding site. Except in the case of CO, the rate of bond making is approximately the same for diatomics as for the bulkier isonitriles.

Patel et al. [42] showed that the equilibrium constants for the binding series of alkyl isonitriles to ferrous Cyt *c'* from *Rhodospirillum molischianum* are in the following order: methyl > ethyl < *n*-propyl < *tert*-butyl < *n*-butyl < amyl < cyclohexyl < *n*-hexyl. They explained that the decrease in the equilibrium constant from MeNC to EtNC provides evidence for a steric interaction of the ligand and the protein. The increase in the equilibrium constant of bulky isonitriles is accounted for by a favourable partitioning of the ligand into a hydrophobic heme coordination site.

The mechanism of the ligand binding with Mb was investigated in detail by Olson and coworkers [43]. Association and dissociation rate constants were measured for O_2, CO, and alkyl isonitrile binding to a set of genetically engineered sperm whale Hbs with site-specific mutations at residue 64 (the E7 helical position). Native His was replaced by Gly, Val, Leu, Met, Phe, Gln, Arg, and Asp using the synthetic gene and expression system. It was indicated that the barrier to isonitrile entry into the distal pocket is primarily steric in nature. A major kinetic barrier for O_2 and CO binding to native Mb may involve disruption of polar interactions between His^{64} and water molecules found in the distal pocket of desoxy Mb.

Further association and dissociation rate constants for O_2, CO, and alkyl isonitriles binding were measured by stopped-flow rapid mixing, conventional flash, and laser photolysis techniques with site-directed mutants of sperm whale Mb to probe the functional role of the highly conserved distal pocket valine residue, Val^{68} (E11) [44]. This amino acid was replaced with Ala, Ile, and Phe. The association and dissociation rate constants for O_2 and isonitrile binding were decreased for the Phe^{68} mutant Mb. These kinetic parameters result in a small change in O_2 affinity and an increase in isonitrile affinity, relative to the native protein. Thus, the large benzyl side chain of phenylalanine at position 68 inhibits

the rate of ligand movement up to and away from the iron atom, but not the final bound state.

The time course of ligand recombination of the Mb from *Aplysia limacina* was measured using photolysis by flashes of 35 picoseconds to 300 nanoseconds by Bellelli et al. [45]. CO shows only bimolecular recombination, O_2 has a small geminate reaction with a half-time of tens of picoseconds. NO has two picosecond relations with half-times of 70 picoseconds, MeNC and EtNC have a geminate reaction with a half-time of 35 picoseconds. It was supposed that there is little evidence to suggest that the reactivity of the heme iron in *A. limacina* Mb is much different from that in sperm whale Mb, and CO shows another mechanism of recombination than O_2, NO, and isonitriles.

Chatfield, Walda & Magde [46] also used the laser flash photolysis to study the rate of escape of O_2, MeNC, and t-BuNC from horse heart Mb. It is surprising that there is little difference between oxygen and the alkyl isonitriles. In the rigid protein approximation the increased bulk of the isonitriles would be expected to give rise to a larger barrier to escape. The slightly larger activation energy for the escape of MeNC as compared to oxygen hints at this effect. On the other hand, the close correspondence between the activation energy for the escape of t-BuNC and oxygen argues against the hypothesis. Their results can be rationalized by assuming that bulkier ligands distort the protein environment surrounding the heme cavity.

The effect of pressure on the recombination kinetics of small ligands binding to sperm whale Mb, protoheme dimethyl ester, and monochelated protoheme was studied using laser flash photolysis by Taube et al. [47]. The volumes of activation observed indicate that in both the protein and the models bond formation is the rate-determining step only for CO, while for O_2 and isonitriles almost no bond formation occurs in the transition state of the observed reaction.

It can be said that using isonitriles in the investigation of the reaction mechanism of ferroheme in the Hbs, Mbs, and Cyts with ligands has helped to clarify it, but it is still very far from being clear. Several investigations [34, 39, 41, 45, 47] show that the mechanism of recombination with ferroheme surrounded by protein is not the same for carbon monoxide and the isonitriles.

On the other hand, it is known that the isonitriles are more or less toxic. Isonitriles sold by Aldrich [48] are classified conventionally into four groups of toxicity: highly toxic, toxic, harmful, and may be harmful (for example diethyl(isocyanomethyl)phosphonate). There is only one paper available on toxicity in which the inhalation LC_{50} values of t-BuNC were determined for rats (710–715 mg/m^3) and mice (356–583 mg/m^3). t-BuNC concentrations in the air show also embryotoxic and anti-spermatogenic effects in animals [49]. The toxicity of the isonitriles is likely based on their reaction with heme iron, but other factors may exist as well. It is also interesting to mention that it is possible to synthesize from isonitriles porphyrines – components of heme [50].

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ISONITRIILID (ISOTSÜANIIDID) KUI MUDELÜHENDID HEMOGLOBIINIDES, MÜOGLOBIINIDES JA TSÜTOKROOMIDES TOIMUVA HEEMI RAUA JA LIGANDIDE VAHELISE REAKTSIOONI MEHHANISMI JA KINEETIKA UURIMISEL

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On antud lühike ajalooline ülevaade hemoglobiinides, müoglobiinides ja tsütokroomides toimuvate isonitriilide ning heemi raua reaktsioonide mehhanismi ja kineetika alastest uurimustest. On näidatud, kuidas põhjaliku uurimistööga jõuti Paulingi hüpoteesist reaalse "tasku" mudelini, mis kirjeldab ligandide ja ferro-heemi vahelise sideme teket. Samuti on tehtud lühikokkuvõte isonitriilide toksilisusest.