Review

Non-enzymatic nitric oxide synthesis in biological systems

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Abstract

Nitric oxide (NO) is an important regulator of a variety of biological functions, and also has a role in the pathogenesis of cellular injury. It had been generally accepted that NO is solely generated in biological tissues by specific nitric oxide synthases (NOS) which metabolize arginine to citrulline with the formation of NO. However, NO can also be generated in tissues by either direct disproportionation or reduction of nitrite to NO under the acidic and highly reduced conditions which occur in disease states, such as ischemia. This NO formation is not blocked by NOS inhibitors and with long periods of ischemia progressing to necrosis, this mechanism of NO formation predominates. In postischemic tissues, NOS-independent NO generation has been observed to result in cellular injury with a loss of organ function. The kinetics and magnitude of nitrite disproportionation have been recently characterized and the corresponding rate law of NO formation derived. It was observed that the generation and accumulation of NO from typical nitrite concentrations found in biological tissues increases 100-fold when the pH falls from 7.4 to 5.5. It was also observed that ischemic cardiac tissue contains reducing equivalents which reduce nitrite to NO, further increasing the rate of NO formation more than 40-fold. Under these conditions, the magnitude of enzyme-independent NO generation exceeds that which can be generated by tissue concentrations of NOS. The existence of this enzyme-independent mechanism of NO formation has important implications in our understanding of the pathogenesis and treatment of tissue injury. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Nitric oxide synthesis; Nitrite disproportionation; Electron paramagnetic resonance; Nitric oxide imaging; Ischemia; Acidosis

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1. Introduction

The gaseous free radical nitric oxide (NO) is generated in biological tissues and is an important regulator of a broad range of functions [1]. It was first shown that acetylcholine induced vascular relaxation is mediated by a labile factor termed endothelial derived relaxing factor [2]. Subsequently, this factor was shown to be NO [3–5]. NO synthesis was first discovered in macrophages, endothelial cells, and neuronal cells [3–6]. An enzyme was identified, nitric oxide synthase (NOS), which synthesizes NO from arginine [7,8]. NO is now known to regulate blood pressure, vascular tone, neural signaling, and immunological function [9–11]. It may also cause cellular injury via reaction with superoxide to form the potent oxidant peroxynitrite [12].

While NOS had been generally considered to be the primary source of NO in biological systems, there have also been reports that enzyme-independent NO generation also occurs. This was initially recognized in the stomach and mouth. It was known that nitrate is concentrated in the saliva and rapidly converted to nitrite by facultative anaerobic bacteria [13]. Benjamin and colleagues reported in 1994 that nitrite is converted to NO under the highly acidic conditions (pH 3) which occur in the lumen of the stomach [14]. They further postulated that this NO is important as a defense against swallowed pathogenic microorganisms. Also, in 1994, Lundberg et al. reported measuring high levels of NO in expelled air from the stomach of humans and demonstrated that this required a functional proton pump [15]. They suggested that measurements of NO in expelled air might be used as a non-invasive method for estimation of gastric acidity. Subsequently in 1995, we observed that nitrite can also be a prominent source of NO in biological tissues under conditions of intracellular acidosis, such as occur following the onset of ischemia as occurs following vascular occlusion [16]. This process was shown to occur following myocardial ischemia as would occur in the clinical setting of heart attack and it was extrapolated that nitrite disproportionation and reduction would be a generalized phenomena in any tissue under conditions of ischemia or shock, where poor perfusion and accompanying acidosis occur.

In this review, we will summarize the work from our laboratory which demonstrates that NOS-independent NO formation occurs in biological tissues and is derived from nitrite. The significance of this process in postischemic cellular injury will also be reviewed. Finally the reaction process of nitrite disproportionation and corresponding rate law of NO formation will be presented which enables consideration of the magnitude of NO formation from nitrite under a broad range of biological conditions.

2. Measurement of NOS-independent NO formation

Since NO is paramagnetic and binds with high affinity to a variety of metal chelates and metalloproteins the distinctive electron paramagnetic resonance (EPR) spectra of these nitrosyl-complexes can serve as a quantitative measure of NO generation [16–18]. The ferrous iron complex of N-methyl-D-glucamine-dithiocarbamate (MGD), Fe(II)-MGD₂ (Fe-MGD), has been shown to be suitable for NO measurements in living tissues [19,20]. This technique was applied to directly measure the NO formation which occurs in the heart following the onset of ischemia.

Studies were performed in rat hearts which were perfused using Krebs bicarbonate buffer bubbled with 95% O₂–5% CO₂ gas at 37°C, with contractile function and flow monitored [21]. Hearts were in-
fused with 1 mM Fe-MGD, subjected to 30 min global ischemia at 37°C, and then rapidly frozen using liquid nitrogen cooled tongs. In normally perfused hearts only a very weak signal of the triplet NO-Fe(II)-MGD complex was seen (Fig. 1A). In hearts subjected to 30 min ischemia, however, a prominent triplet NO adduct signal was observed over the magnetic field range from 3240 to 3290 G with a central g value of 2.04 and hyperfine splitting of 12.5 G (Fig. 1B). Additional intrinsic signals in the g = 2.008–2.000 region have been reported just up-field from the NO triplet and correspond to the positive deflections at the high field end of the spectra [22]. Over a 10-fold increase in the intensity of the NO triplet was seen after 30 min ischemia compared to that seen in control hearts.

At a concentration of 1 mM, the NOS blocker N-nitro-l-arginine methyl ester, l-NAME, totally inhibits the conversion of arginine to NO and citrulline [23]. Infusion of 1 mM l-NAME into normally perfused hearts for 5 min was also sufficient to cause maximum depression of coronary flow. Even though l-NAME totally blocked NOS-mediated vasorelaxation, the formation of the NO triplet signal in hearts after 30 min ischemia was only partially inhibited, with only a 60–80% decrease observed in a series of five hearts (Fig. 1C). In additional experiments using higher concentrations of l-NAME, up to 5 mM, or N G-monomethyl-l-arginine, no further inhibition was observed. This lack of total inhibition of NO formation with blockade of NOS suggested that there may be an enzyme-independent pathway of NO formation.

NO formation can also be directly measured via the binding to intrinsic metallo-heme centers within the tissue such as myoglobin. The binding of NO to these proteins gives rise to a unique EPR spectrum with axial symmetry and a characteristic triplet splitting due to the hyperfine coupling of the nitrogen nucleus of directly bound NO [17,19]. These complexes are inherently oxygen labile and would be best observed at low oxygen tensions as occur with prolonged ischemia. Therefore, experiments were
performed measuring nitrosyl-heme formation in control heart tissue and tissue subjected to prolonged ischemia. In control hearts, no significant nitrosyl-heme signal was observed, however, in ischemic tissue after periods of 1–12 h increasingly prominent nitrosyl-heme triplet signals were observed further demonstrating that increased NO formation and accumulation occurs during myocardial ischemia (Fig. 2A,B). Pretreatment of hearts with either 1 or even 5 mM l-NAME prior to the onset of ischemia only partially decreased these NO triplet signals with only a 30–50% decrease seen after 4–8 h of ischemia (Fig. 2C). Thus, these experiments further support the existence of an enzyme-independent pathway of NO generation.

3. Demonstration that NOS-independent NO is derived from nitrite

During myocardial ischemia, marked intracellular acidosis occurs along with severe hypoxia leading to a highly reduced state that could cause the reduction of nitrite to NO [24,25]. To determine if nitrite, NO$_2^-$, is reduced during ischemia to form NO, experiments were performed measuring NO with Fe-MGD in hearts subjected to ischemia in the presence of isotopically labeled $^{15}$NO$_2^-$. Since $^{15}$N has a nuclear spin of 1/2, doublet hyperfine splitting will be observed in the EPR spectra of NO complexes instead of the triplet splitting observed for the natural abundance $^{14}$NO$_2^-$. In the presence of $^{14}$NO$_2^-$, a prominent triplet nitroso-heme signal is seen (A), while in the presence of $^{15}$NO$_2^-$, a doublet signal is observed (B), indicating that the NO formation was directly derived from nitrite.
hearts subjected to ischemia after labeling with 100 μM NO\textsubscript{3}\textsuperscript{2}, the signal was only 10-fold above that in the absence of added nitrite. These experiments demonstrate that nitrite is reduced to NO in the ischemic heart.

Experiments were also performed measuring nitrosyl-heme formation in ischemic heart tissue from hearts labeled with 1 mM 15NO\textsubscript{3}\textsuperscript{2}. A prominent doublet nitrosyl-heme signal was seen due to the formation and binding of 15NO\textsubscript{2} to these proteins, further confirming that NO is generated from nitrite in heart tissue subjected to long periods of ischemia (Fig. 3B, right). With 14NO\textsubscript{3}\textsuperscript{2} a similar magnitude triplet NO signal was observed (Fig. 3A, right).

To determine if there is sufficient nitrite present in the heart for nitrite-mediated NO formation to occur during ischemia, experiments were performed to measure the concentration of nitrite within the heart prior to ischemia. Measurements were performed using a Sievers 270B nitric oxide analyzer [25]. Heart extracts were prepared and in a series of five hearts the nitrite concentration was 12 ± 5 μM. Thus, there is a large pool of nitrite present at the onset of ischemia.

The rate of reduction of nitrite to NO is pH dependent, with increased reduction with increasing acidosis. To correlate the effect of myocardial pH on this process of NO formation, intracellular pH was measured by in vivo 31P-NMR and these results were correlated with in vivo EPR measurements of NO formation in the whole heart using Fe-MGD. These in vivo magnetic resonance techniques enable real time measurement of the time course of changes in pH and NO within the heart [24,26]. After 10 min of ischemia, the myocardial pH decreased to 5.9 and by 20 min to a value of 5.5 (Fig. 4). Prior to and for the first 6 min of ischemia, there was no measured...
NO signal. After 7–8 min of ischemia, this signal was first observed and then it rapidly increased over the next 30 min accompanying the decrease in tissue pH (Fig. 4). The threshold pH value at which NO generation was first seen was approximately 6.0.

4. Imaging nitrite-derived NO

With the development of EPR imaging (EPRI) methods and low frequency EPR instrumentation, it has become possible to spatially map the presence, quantity, and location of free radicals and paramagnetic species in both in vivo and ex vivo biological tissues [27]. The ability to image NO production with EPRI in biological tissues was first reported in the ischemic brain [28] and subsequently studies were reported mapping NO formation in the ischemic heart [29]. With combination of EPRI and the use of isotope tracer methods, the precise metabolic pathway of NO formation can be mapped. With the use of $^{15}$N nitrite, spatial mapping of nitrite-derived NO was performed in the ischemic heart [29].

A series of rat hearts were loaded with Fe-MGD and $^{14}$N or $^{15}$N nitrite and subjected to global no flow ischemia. After 60 min three-dimensional projection data were acquired and image reconstruction performed. Fig. 5 shows 3D images obtained in these hearts. Both the $^{14}$N- and $^{15}$N-labeled hearts exhibited similar images of NO distribution. The $^{15}$NO image, however, is somewhat sharper due to enhanced sensitivity and better resolution of the hyperfine doublet structure. The images clearly show that NO is formed throughout the left ventricular (LV) myocardium. The NO image enabled visualization of the external shape of the epicardium, right ventricular (RV) myocardium and internal endocardial surface of the LV and LV chamber. The concentrations of NO in the RV, however, were much lower with levels only 20–25% of those seen in the LV wall.

To understand the spatially resolved time course of NO generation in the ischemic heart, EPRI of NO formation was performed as a function of ischemic duration. A series of images were performed every 5 min during ischemia for a total period of 60 min. Two representative slices, a longitudinal and a transverse, cut through the LV are shown in Fig. 6. It was observed that the NO concentration derived from nitrite gradually increases as a function of the duration of ischemia throughout the LV myocardium.

5. Evaluation of the role of nitrite-derived NO in postischemic injury

It was previously shown that NO generated from
nitric oxide synthase can result in a loss of recovery of contractile function after reperfusion [17]. To evaluate the relative importance of enzyme-dependent versus enzyme-independent NO generation in the pathogenesis of postischemic injury, hemodynamic studies were performed in hearts which were subjected to 30 min of global 37°C ischemia followed by 45 min of reperfusion. The four groups were as follows: (1) untreated control; (2) pretreated with 1.0 mM L-NAME for the 5 min prior to the onset of ischemia to block nitric oxide synthase; (3) treated with 10 μM oxyhemoglobin 1 min prior to ischemia as well as the first 5 min of reflow, to scavenge NO generated by either pathway; and (4) pretreated with both 10 μM nitrite and 1.0 mM L-NAME, to determine if NO$_3^-$ can modulate the protective effects of enzyme inhibition. Left ventricular pressures and heart rate were measured using a left ventricular balloon [13,18]. The graph shows the recovery of the product of left ventricular developed pressure and heart rate (RPP), an index of cardiac contractile work, expressed as % of baseline preischemic values. While inhibition of enzyme-dependent NO formation resulted in cardioprotection, further protection was seen in the presence of oxyhemoglobin which scavenges NO from both pathways. It was observed that supplementation of nitrite within the heart abolished the protection seen with enzyme inhibition.

The time course of the recovery of rate pressure product, a measure of contractile work, are shown for the first four groups (Fig. 7). As reported previously [17], L-NAME-treated hearts exhibited significantly higher recovery of contractile function than untreated control hearts ($P<0.01$, Fig. 7). Oxyhemoglobin-treated hearts, however, exhibited an even higher recovery of contractile function than the L-NAME-treated hearts ($P<0.04$, Fig. 7). While, L-NAME only blocks NO formation from nitric oxide synthase, oxyhemoglobin scavenges NO formed from either pathway. Thus, this data suggests that enzyme-independent NO formation contributes to the process of postischemic injury in the reperfused heart. To determine if further enhancing NO production from nitrite would reverse the protection afforded by L-NAME, experiments were performed in which hearts were also infused with 10 μM nitrite during the 5 min immediately prior to ischemia. It was observed that this nitrite loading further increased postischemic injury and almost totally blocked the protective action of L-NAME (Fig. 7).

In order to further determine the relative magnitude and functional importance of NO generated from each pathway, 3–4 additional hearts were studied in each of the six groups for EPR measurements of NO generation at end ischemia using Fe-MGD and these results were correlated with the final recovery of contractile function observed after reperfusion (Fig. 8). Preischemic infusion with arginine did not significantly increase NO generation and did not alter the recovery of contractile function. This probably indicates that tissue arginine concentrations remained well above the $K_m$ of nitric oxide synthase even in the absence of arginine infusion. L-NAME pretreatment decreased nitric oxide generation by about 65% and resulted in approximately a two-fold increase in the recovery of contractile function. With preischemic infusion of nitrite in addition to L-NAME, NO generation markedly increased and functional injury was restored. With preischemic loading of nitrite, NO generation was increased by 2–3-fold above levels seen in the untreated controls, and marked impairment of contractile function was seen; however, the function was only slightly lower than in the untreated controls. In both of these groups, very severe functional injury was present with more than a 90% loss of contractile function.
Because of the severe, near-complete, extent of this injury, further increases in NO generation from nitrite may only result in the small further decreases in functional recovery which were seen. Alternatively, since it has been hypothesized that NO-mediated injury is due to its reaction with superoxide to form the potent oxidant peroxynitrite [12], it is possible that the magnitude of tissue injury could reach a maximum value when the concentrations of NO approach those of superoxide. With oxyhemoglobin treatment, NO was almost totally quenched with more than a 3-fold increase in the recovery of contractile function above the values observed in untreated controls. Thus, this data further confirms the presence and functional importance of enzyme-independent NO generation. In general, it is clear from these studies that both enzyme-dependent and enzyme-independent NO formation is increased during ischemia and that the magnitude of this NO generation correlated with the severity of functional injury observed upon reperfusion.

These results demonstrate that enzyme-independent NO formation not only contributes to posts ischemic injury, but it may also reverse the protective effects of NOS blockers. Of note, we observed in isolated perfused hearts that significant washout of tissue nitrite occurs during perfusion and that the nitrite concentrations decrease by more than 3-fold from 12.5 to 3.9 μM during the 15-min period of initial perfusion. Thus, the enzyme-independent pathway of NO formation would be expected to be even more prominent in vivo than in these isolated heart studies. In addition, with ischemic durations longer than 30 min, we observe that the magnitude of NO formation from this pathway is greatly increased.
6. Is NOS-independent NO formation truly enzyme independent?

While the studies summarized above definitively demonstrate that NO generation occurs in ischemic tissues from nitrite via a NOS-independent pathway, it was questioned whether this process was independent of other enzymes as well. Meyer noted prior reports that cytochrome \( c \) oxidase could also reduce nitrite to NO and questioned whether this was a source of NOS-independent NO generation [30]. Our original studies demonstrated that nitrite can be directly reduced in the ischemic heart to form large amounts of NO. While inhibitors of NOS did decrease NO formation, blockade of the enzyme (NOS) at the onset of ischemia was not sufficient to totally prevent the formation of NO. We observed that nitrite is present in the tissue in micromolar concentrations and is converted to NO under the acidic and highly reduced conditions which occur during ischemia. With long periods of tissue ischemia progressing to necrosis this mechanism of NO formation was found to be the major source of NO generation.

In considering the role of other enzymes, it was pointed out that correlative EPR measurements of NO and \(^{31}\)P-NMR measurements of intracellular pH demonstrate that the magnitude of NO generation from nitrite increases as the pH of the tissue decreases [31]. After only 30 min of ischemia, myocardial pH decreases to less that 5.5; however, marked NO production continued for several hours after this, as evidenced by marked increases in nitroso-heme formation. It is questionable whether cytochrome oxidase or other enzymes would be able to function at these very low pH values. However, nitrite itself is readily converted to NO at these values of pH. Therefore, one does not have to invoke the presence of cytochrome oxidase or any other enzyme to explain the marked NO generation which occurs.

To further explore the pathway of NO generation from nitrite and the role of non-NOS enzymes in this process, we performed in vitro experiments to confirm whether acidification of nitrite in the absence of any enzyme would result in NO formation similar to that in ischemic tissues. EPR spin trapping measurements using the NO trap Fe-MGD were performed [31]. While preparations of nitrite at pH 7.4, only give rise to a trace NO signal; preparations acidified to pH 5 exhibit a very large characteristic NO triplet signal (Fig. 9). This confirms that acidification of nitrite in the absence of any enzyme results in marked NO generation.

To further explore whether the enzyme cytochrome oxidase was required for nitrite-mediated NO formation in the ischemic heart, experiments were performed in which hearts were preinfused with 5 mM sodium cyanide for 5 min prior to the onset of ischemia, in a manner similar to that previously demonstrated to block cytochrome oxidase [32]. These hearts were then loaded with Fe-MGD and nitrite and subjected to 30 min of global ischemia at 37°C. In these cytochrome oxidase-blocked hearts, a large characteristic NO adduct signal was observed similar to that in unblocked hearts (Fig. 10). Thus, NO generation in the ischemic heart can occur independent of cytochrome oxidase.

In spite of these observations, cytochrome oxidase or other myocardial heme enzymes or proteins could potentially influence the in vivo process of NO formation from nitrite. Oxygen consumption by these enzymes is the fundamental mechanism which results in hypoxia and a highly reduced state in ischemic tissues. Therefore these enzymes clearly have a criti-
ical role in the process of nitrite-mediated NO generation and accumulation in ischemic tissues. These enzymes could also be important targets of NO or peroxynitrite-mediated damage. It will be important in future studies to fully delineate the role of cytochrome oxidase and other heme enzymes as a source or a target of nitric oxide and nitric oxide-mediated injury.

7. Reaction process and rate law of nitrite disproportionation

Recently, we have performed EPR and chemiluminescence studies to characterize the magnitude of the nitrite disproportionation process [33]. The reaction process, based on the reaction pathways of nitrogen oxides and related species (Fig. 11), was proposed and the rate law of NO formation was derived. This enables comparison of the magnitude of this process to that of in vivo NO production, and thus enables determination of the quantitative importance of this pathway compared to that from NOS or other sources. It also enables assessment of whether NO is derived directly from nitrite disproportionation or if nitrite reduction occurs and reducing equivalents are required.

It is known that nitrite under acidic conditions exists in equilibrium with nitrous acid and its concentration depends on the acidity of the solution:

$$\left[\text{HNO}_2\right] = \frac{[\text{NO}_2^-][\text{H}^+]}{[\text{H}^+] + K_\alpha}$$

$$\left[\text{NO}_2^2\right] = \frac{[\text{NO}_2^-][K_\alpha]}{[\text{H}^+] + K_\alpha}$$

where $[\text{NO}_2^-]$ is the initial concentration of nitrite added to the reaction mixture; and $K_\alpha$ (pK = 3.4) is the dissociation constant of nitrous acid [34]. Nitrous acid exists in equilibrium with nitrosonium ion [35] or nitrosonium like intermediates [36]. On Fig. 11 we named this as $\{\text{NOOH}\}$, for such an isoform of nitrous acid. In addition, nitrate exists in equilibrium with dinitrogen trioxide that can decompose to NO and NO$_2$. NO$_2$ is able to dimerise to N$_2$O$_4$ and it interacts with water to form nitrite and nitrate. This nitrite in solutions exists in complex equilibrium with different kinds of oxygen-containing derivatives. To avoid effects of final products and intermediates, we used the method of initial rates to derive the rate law. Analysis of the rate of NO formation as a function of initial nitrite concentration showed that at low nitrite concentrations the reaction is second order in nitrite [33]. Increasing the concentration gradually dropped the order of the reaction to first. The formation of NO was also pH dependent. At pH 4–7, the reaction is first order in [H$^+$], while at lower pH, the reaction becomes independent of [H$^+$], zero order.

The observed effect of nitrite and H$^+$ ion concentration on the rate of NO production could not be described by the rate law directly calculated from the equation of nitrite (nitrous acid) disproportionation. The best fitting was found for the reaction scheme:

$$\text{HNO}_2 \overset{k_1}{\underset{k_{-1}}{\leftrightarrow}} \{\text{NOOH}\} \overset{k_2}{\rightarrow} \text{NO} + \text{NO}_2$$

$$\text{N}_2\text{O}_3 + \text{OH}^- \overset{k_1}{\rightarrow} \text{NO} + \text{NO}_2$$

To derive the rate law for this postulated scheme, two approximations were considered. The step of nitrous acid formation, as noted above, is considered to be much faster than the others, and the intermediate $\{\text{NOOH}\}$ and N$_2$O$_3$ were considered so reactive that they do not accumulate at an appreciable level compared to nitrite. The combination of the steady-state and the prior-equilibrium approximations was applied to describe the rate law, using an approach similar to that described [11]. The expression derived for the rate law of the reaction is as follows:
where

\[ K_\beta = \frac{K_{-1}}{K_2} \]

Fitting gives values \( K_1 = 9.3 \times 10^{-4} \), \( K_\beta = 9.3 \times 10^{-4} \).

This model yields the graphs illustrated in Fig. 12, which as shown accurately predict the rate of NO formation from nitrite over the range of pH values from 3 to 7 and nitrite concentration from 1 \( \mu \)M to 10 mM.

Substitution of the pH and nitrite concentration values that occur in normal tissues of 7.2–7.4 and 10–50 \( \mu \)M, respectively, a rate of NO production of 0.05–1 pM/s is predicted. This is on the same order of magnitude as the values reported for NO production from the kidney of 85 fmol/min/g which corresponds to 1.4 pM/s [37].

Under ischemic conditions where pH can fall to values as low as 5.5, a much higher rate of 4–100 pM/s is predicted. This would result in accumulation of 15–360 nM concentrations of NO during 60 min of ischemia. For comparison, basal NO release into the coronary effluent of guinea pig hearts at flows of 10 ml/min has been reported to be 161 pmol/min [38], which corresponds to 1.3 nmol/s/kg. It has been reported from studies of rat heart homogenates that NO production from maximally activated NOS is 1 pmol/min/mg of protein or 1.5 nmol/s/kg tissue [39]. Thus, the production of NO from nitrite disproportionation under ischemic conditions can correspond to as much as 5–10% of the maximum production that could occur from NOS. Furthermore, it has been shown that at the low pH values which occur during ischemia, NOS loses its catalytic activity [39]. Therefore NO formation from nitrite would be of particular importance in this setting.

The rate of NO production from nitrite can be altered by tissue-dependent factors. Reducing equivalents which accumulate during ischemia could further increase the rate of NO formation. Chemiluminescence measurements in the presence of aliquots of ischemic tissue homogenates demonstrate that the reducing equivalents present increase the rate of NO generation 40–50-fold above that due to disproportion alone [33]. The reducing equivalents in ischemic tissue were observed to be highly oxygen labile and were not abolished by heating to 80°C for 15 min which is sufficient to denature most enzymes. Thus, while these equivalents may be enzyme derived, the process of nitrite reduction itself does not appear to require intrinsic enzyme function.

8. Conclusion

Studies from our laboratory and others have shown that enzyme-independent NO formation occurs in biological systems under acidic conditions and is further increased in the presence of a highly reduced metabolic state. The substrate source of this NO is nitrite, rather than the NOS substrate, L-arginine. Under hypoxic conditions, this NO accumulates in tissues and binds to a variety of cellular heme proteins. This mechanism of NO formation is a generalized phenomenon which can occur in all biological systems.
biological tissues. In important disease states, such as ischemia or shock, where acidosis and marked hypoxia occur, this pathway becomes the major source of NO and the magnitude of NO generation can be much greater than that which would be formed by normal tissue concentrations of NOS. The existence of this enzyme-independent mechanism of NO formation has important implications in our understanding of the pathogenesis and treatment of disease.

References

[29] P. Kuppusamy, P. Wang, A. Samouilov, J.L. Zweier, Spatial mapping of nitric oxide generation in the ischemic heart


