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Hypothesis

NO, thiols and disulfides

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The chemical nature of the messenger molecule, nitric oxide (NO), and especially its reactivity towards thiol groups and disulfides, could explain, at least partly, its intervention in so many different biological processes. NO can be regarded as the smallest molecule suitable for electron transport in biological systems. The S-nitrosation reaction and its reverse reaction represent the most convenient general way to store, to transport and finally to release NO. Nitric oxide is also particularly convenient for playing a role in interconversions of thiol groups and disulfides in chain radical or oxidation–reduction processes, and to be subsequently engaged in complex sequences of reactions accounting for different biological situations.

NO; Nitric oxide; Thiol; Disulfide

The recently discovered new messenger molecule, nitric oxide (NO) – or some nitric oxide carrier, vide infra – has been shown to play a major role in at least three biological processes: vessel smooth muscle relaxation, neurotransmission modulation and macrophage cytotoxicity [1–5].

It may appear surprising that so simple a molecule could serve in so many different biological processes. NO is one of the smallest molecules known, a gas with limited water solubility, an unstable free radical species highly reactive towards atoms or molecules containing unpaired electrons such as molecular oxygen, superoxide anions or protein-bound metals.

Enzymic systems responsible for NO formation seem largely distributed, at least in the animal kingdom. NO has been shown to be produced from L-arginine and molecular oxygen via an as yet to be completely determined sequence of reactions controlled by an NO synthase [6,7]. According to the different cell types, at least two forms of that enzyme exist. Such an NO synthase is present in hemocytes of the American horseshoe crab (*Limulus polyphemus*) indicating that NO formation from L-arginine is a biochemical pathway of early evolutionary origin [8]. That also suggests that NO bioreactivity could follow very 'ancient' chemical pathways based upon relatively simple reactions.

A first possible answer to what makes NO so special was given by Traylor et al. [9]. NO is presented as a very peculiar ligand for heme and non-heme iron proteins especially with regard to its unusual binding affinity to various iron(II) porphyrins. Another point is that NO binds iron better without proximal base, suggesting a scenario involving imidazole release and participation for the mechanism of guanylate cyclase activation by NO [10].

To the question "why NO?", we propose an additional answer with regard to the chemical nature and hence the reactivity of that molecule, which could also, at least partly, explain its intervention in so many different biological processes. NO is a biatomic radical-molecule bearing an unpaired electron, which can be regarded as the smallest molecule suitable for electron transport. Moreover, in appropriate conditions, NO can react with thiol groups and disulfides to form *S*-nitroso compounds, in a more or less reversible manner [11]; it can also be subsequently engaged in complex chain radical or oxidation–reduction processes.

As a very simple free radical species, NO can be considered as one of the ways selected by nature for transporting electrons from one point to another of biological systems. Halliwell [12] recently reported an interesting hypothesis due to Baldwin et al. [13] and confirmed by some experiments, according to which in living organisms thiols, thiolates and disulfides are interdependent and constitute elements of an electron transfer system alongside with metalloproteins and some amino acids involved in peptides or proteins. Owing to its chemical nature, NO can undoubtedly take part in these types of biochemical processes and facilitate such electron transfer mechanisms.

Due to the relative instability of the formed *S*-nitroso compounds, the S-nitrosation reaction has received much less attention than the similar O-nitrosation reaction [11]. In appropriate conditions, NO is therefore able to react directly with thiols or disulfides to form *S*-nitroso compounds, for example with low p*K*s and/or conveniently sterically hindered thiol groups. The re-

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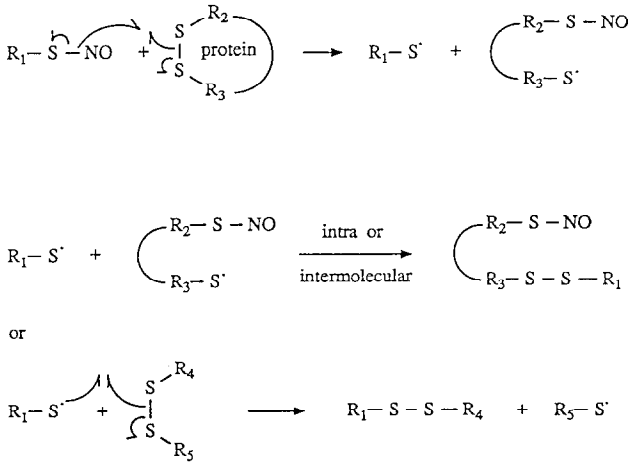

Fig. 1. Examples of reactions of *S*-nitrosospecies involving reversible thiol-disulfide exchanges.

lease of NO from a *S*-nitroso compound – under slightly different conditions – is much easier. The S-nitrosation reaction and its reverse reaction represent then the most convenient general way to store, to transport and finally to release NO. Due to the large variety of their possible chemical structures, *S*-nitroso compounds form a family of NO-carrier compounds whose every member could be suitable for a particular biological effect. Such a *S*-nitroso adduct of serum albumin was recently evidenced as a NO reservoir in mammalian plasma [14].

Interconversions of thiols and disulfides in oxidation–reduction processes are commonly used in biology not only to improve protein structural stability but also to ensure enzymatic catalysis, transport of reducing equivalents or metabolic regulation [15]. Such reactions can also involve suitable electron donors and acceptors. Due to its reactivity NO is particularly convenient to play a role in such sequences of reactions. The *S*-nitroso species formed from a thiol or a disulfide group and NO can, either give back a thiol by reduction and consequently release NO, or react with another disulfide group to give a new *S*-nitroso species, on the one hand, and a new *S–S* group on the other. The process can be repeated. Termination of such chain processes can occur in different ways (see Fig. 1 for some examples of such reactions).

The important point is that NO could be stored and released in reactions involving reversible thiol–disulfide exchange and that it can be then regarded as an inducer of protein rearrangement in a durable although reversible manner, depending for example upon redox conditions. NO can then be involved in various processes such as activation or de-activation of disulfide bond-containing enzymes or receptors, via their rearrangement to active or inactive forms. Some allosteric situations can also certainly be accounted for.

Recently, nitric oxide has been claimed to account for the retrograde messenger involved in the long term potentiation phenomenon (LTP) [16]. We hypothesize that cysteine-containing proteins could be considered at least partly as the biological material used for the storage of information in the brain and that NO plays a central role in modifying in a reversible manner the conformations of these proteins and possibly also in forming new networks through intermolecular disulfide bridges formation.

It is worth noting that the effect of NO on the redox modulatory site of the NMDA receptor-channel complex was quite recently hypothesized to involve *S*-nitroso derivatives and disulfide bonds formation [17]. The mechanism of guanylate cyclase activation by NO can also include, at least partly, similar reactions [18].

There is no doubt that the particular chemical nature of NO and especially its reactivity towards thiol groups and disulfides are the source of its unusual and crucial role in so many and different areas of biology. Many studies will therefore be still necessary to explore all the aspects of this ubiquitous molecule whose biochemistry is still poorly understood [19].

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