The Metabolism of Proline as Microenvironmental Stress Substrate$^{1-3}$

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Abstract

Proline, a unique proteogenic secondary amino acid, has its own metabolic system with special features. Recent findings defining the regulation of this system led us to propose that proline is a stress substrate in the microenvironment of inflammation and tumorigenesis. The criteria for proline as a stress substrate are: 1) the enzymes utilizing proline respond to stress signaling; 2) there is a large, mobilizable pool of proline; and 3) the metabolism of proline serves special stress functions. Studies show that the proline-utilizing enzyme, proline oxidase (POX)/proline dehydrogenase (PRODH), responds to genotoxic, inflammatory, and nutrient stress. Proline as substrate is stored as collagen in extracellular matrix, connective tissue, and bone and it is rapidly released from this reservoir by the sequential action of matrix metalloproteinases, peptidases, and prolidase. Special functions include the use of proline by POX/PRODH to generate superoxide radicals that initiate apoptosis by intrinsic and extrinsic pathways. Under conditions of nutrient stress, proline is an energy source. It provides carbons for the tricarboxylic acid cycle and also participates in the proline cycle. The latter, catalyzed by mitochondrial POX and cytosolic pyrroline-5-carboxylate reductase, shuttles reducing potential from the pentose phosphate pathway into mitochondria to generate ATP and oxidizing potential to activate the cytosolic pentose phosphate pathway. J. Nutr. 138: 2008S–2015S, 2008.

Introduction

The metabolic pathways for proline were first characterized by Elijah Adams and Harold Strecker beginning in the mid-1950s (1–3). It was previously recognized that proline was an unusual amino acid, being the only proteogenic secondary amino acid (1,3). With its $\alpha$ nitrogen contained within a pyrrolidine ring, proline is not a substrate for the usual amino acid-metabolizing enzymes, the decarboxylases, aminotransferases, and racemases. Instead, a family of proline-metabolic enzymes evolved with their own tissue and subcellular localization and mechanisms of regulation. Because proline metabolism is distinct from that of primary amino acids, it can play a regulatory role or, alternatively, its metabolism can be reserved for special physiologic or pathophysiologic situations (2). The understanding of these special functions and their mechanisms has made significant advances during the last decade.

The oxidized congener of proline, $\Delta^1$-pyrroline-5-carboxylate (PSC)$^6$, which is in tautomeric equilibrium with glutamic-$\gamma$-semialdehyde, is in a strategic location in intermediary metabolism (Fig. 1). Lying between glutamate and ornithine, PSC serves as an obligate carbon bridge between the 2 major metabolic cycles, the tricarboxylic acid (TCA) cycle and the urea cycle (2). Proline can be derived from dietary proteins and from the degradation of endogenous proteins, but the final release of proline requires a specific dipeptidase, prolidase, which can hydrolyze the peptide bond constrained within the pyrrolidine ring. Proline can be biosynthesized from glutamate and ornithine (4), making it nutritionally nonessential, but as has been previously mentioned (2), the selective preservation of biosynthetic pathways may relate to endpoints more important than simple supply of that amino acid as substrate for protein synthesis (2). Recognizing that PSC is not only the committed precursor of

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$^6$ Abbreviations used: ECM, extracellular matrix; FAD, flavine adenine dinucleotide; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; PSC, $\Delta^1$-pyrroline-5-carboxylic acid; POX, proline oxidase; PRODH, proline dehydrogenase; ROS, reactive oxygen species; TCA, tricarboxylic acid; TZD, thiazolidinedione.
proline but also its immediate degradative product, we considered that this relationship may be metabolically important. In fact, we showed that these interconversions constitute a catalytic cycle transferring reducing potential into mitochondria and the cycling of proline-P5C participates in a metabolic interlock with the pentose phosphate pathway (2).

The proline cycle

The central enzyme in the proline cycle is proline oxidase (POX) a.k.a. proline dehydrogenase (PRODH). Confusion has arisen in the nomenclature due to the history of the molecular discoveries. Recently, a consensus was reached by a number of laboratories (2nd International Symposium on Proline Metabolism in Health and Disease, September 10–11, 2007, NCI-Frederick, Frederick, Maryland, see Amino Acids, August 2008) that the first enzyme of the proline degradative pathway would be designated POX or PRODH (POX/PRODH). The gene, however, would be referred to as PRODH. The first enzyme of the hydroxyproline degradative pathway, encoded by a distinct gene on a different chromosome and without activity crossover with POX/PRODH, has been the basis for the confusion. It was decided according to the current nomenclature. The catalytic mechanism involves the transfer of electrons from substrate proline to flavine adenine dinucleotide (FAD) with cytochrome c as the subsequent carrier into the electron transport chain. Thus, proline is a direct substrate for the generation of ATP (5,6).

Recently, it was shown that POX/PRODH can reduce oxygen to produce superoxide autogenously. Thus, the trivial name, proline oxidase, is a propos for this enzyme, at least in terms of the monofunctional catalytic mechanism.

When the proline cycle was proposed as a metabolic interlock to shuttle NADPH-reducing equivalents into mitochondria in the form of proline, thereby converting the pentose phosphate pathway into an ATP-generating metabolic pathway (5,10,11), critics disparaged the contribution to bioenergetics as quantitatively trivial compared with that of the TCA cycle or even the glycolytic pathway. Recent emphasis on metabolism in cancer (12,13) emphasizes Otto Warburg’s discovery (14) that oxidative phosphorylation and the TCA cycle are dysfunctional in tumors. Thus, proline as a substrate may very well have its metabolic niche in certain disease states.

Proline as nutrient in the diet or for interorgan transfers

As nutrients, proline and hydroxyproline are not especially abundant in the human diet. For a vegetarian diet, the intake of proline and hydroxyproline is even lower. Convincing correlation between dietary intake of proline and hydroxyproline and disease risk is lacking with the exception that populations with a high intake of red meat (presumably the intake of proline and hydroxyproline was high (15), but it was not quantified) may have a slightly increased risk for breast cancer (16). Overall, dietary perturbation of proline and hydroxyproline has no major effects. However, the interaction of dietary proline and hydroxyproline with pharmacologic intervention remains an intriguing possibility. A case in point is the use of inhibitors of matrix metalloproteinases (MMP) as an antitumor agent. Based on the activation of MMP by a variety of tumors and the participation of MMP in invasion and metastases, drugs were designed to inhibit these enzymes. Although the preclinical studies were very successful in retarding tumor growth and metastasis (17), the clinical trials were disappointing (18). The marked differences between animal diets for these studies, i.e. formulated diets (17) containing no animal products (thus little hydroxyproline), and the ad libitum diets during the clinical trials may be a factor. This difference will be discussed in a subsequent section.

Proline/hydroxyproline does not participate significantly in interorgan transfers of substrates. The main paradigm of circulating substrates involves alanine (19) and glutamine (20). Alanine participates in gluconeogenesis and glutamine in the transfer of carbons from muscle to liver and kidney in the postabsorptive state.
The latter plays an important role in the physiologic regulation of acid-base balance and the delivery of nitrogen to the kidney to be excreted as ammonium chloride (21).

An “out of the box” consideration of nutrients
But with newer concepts of metabolism in physiologic and pathophysiologic states, nutritional metabolism must consider more than dietary sources and circulating interorgan transfers. Clinical therapeutic approaches are also relevant. For example, postsurgical parenteral nutrition includes not only energy substrates but also a complement of amino acids, glutamine, vitamins, and lipids (22). Parenteral nutrition allows delivery of nutrients to tissue sites without gastrointestinal absorption and portal transport, the route for dietary nutrients.

Additionally, current technology allows for selective delivery by catheterization or percutaneous delivery of various agents (23). The developing field of nanotechnology makes possible delivery of specific agents in nanoparticles targeted by surface receptors to designated sites (24). Although these methods remain experimental at present and are focused on rather than nutrient delivery, they are mentioned here because previously unrecognized metabolic roles for certain nutrients may stimulate innovation to develop novel approaches for local nutritional supplementation.

In disease states, the microenvironment has received considerable attention (25) and may be the critical arena in which nutrients must be considered. When a disease process includes isolation of tissues from their blood supply, whether it is the hypoxic myocardium, inflammatory nodule, hemorrhagic cerebral tissue, or malignant tumor, the nutrition within that microenvironment may be critically important in producing tissue damage, promoting healing, or, in the case of malignant tumors, expanding invasion and metastasis (26). In this setting, proline as a substrate in the diseased microenvironment has not been appreciated and this role is the subject of this review. Because the author’s own research has been in the context of cancer, proline will be considered as a substrate primarily in the context of carcinogenesis. However, the contributions of proline as a substrate may be generalized to other disease areas.

Criteria for microenvironmental stress substrates
The advances over the last 6–7 y have persuaded us that proline is a microenvironmental stress substrate. Although the magnitude of the bioenergy generated from proline is much less than that from the TCA cycle or even from glycolysis, the availability of energy from proline may be critical for survival when other substrates, e.g. glucose and glutamine, are unavailable. In a situation where stress is overwhelming, the bioenergetics of proline can be used for programmed cell death. However, for the proline metabolic system to be considered as a special stress responder, the evidence must be persuasive in satisfying the following criteria: 1) the metabolic system for the substrate must respond to stress signals with specific mechanisms for its up-regulation; 2) there is a mobilizable reservoir of the substrate; and 3) the metabolism of the substrate serves special cellular functions.

Responses of the proline metabolic system to stress
Recent advances in proline metabolism were given impetus by the serendipitous finding by Polyak et al. (27) that PRODH, the gene encoding POX/PRODH, is a p53-induced gene. Working in Dr. Bert Vogelstein’s laboratory, they used serial analysis of gene expression to screen the gene targets of p53, a critical cancer suppressor gene. Of 7202 genes monitored, PRODH was 1 of 14 genes highly induced (>7-fold) and designated to be p53-induced gene 6. This finding caused considerable excitement in our laboratory at NCI-Frederick and in the laboratory of Dr. David Valle at Johns Hopkins. Our laboratories have collaborated over the years and our mutual interest in proline was stimulated by the arrival at Hopkins of Chien-An Andy Hu as a postdoctoral fellow. Dr. Hu worked on plant proline metabolic enzymes at Ohio State University. Dr. Hu and Dr. Jian Yu of the Vogelstein laboratory made a POX/PRODH expression vector controlled by a tet-off promoter and obtained stable transfectants in DLD-1 colorectal cancer cells. Steve Donald and Xiao-Ya Sun in my laboratory soon showed that overexpression of POX/PRODH produced proline-dependent reactive oxygen species (ROS) and that the proline-dependent ROS were generated with cytotoxic stress in p53-expressing cells but not in p53 nulls (28). Furthermore, the p53-dependent upregulation of POX/PRODH, as documented by enzyme activity and enzyme protein, was reproduced in a number of cell lines. The resultant apoptosis in a proline-dependent fashion was reported at the American Association for Cancer Research meeting in 2001 by Andy Hu (29). Thus, POX/PRODH expression was clearly upregulated by genotoxic stress. Its consequences will be more completely described subsequently (see below).

To elucidate further the mechanisms of regulation of POX/PRODH, Jui Pandhare and Sandra Cooper developed a PRODH promoter-luciferase reporter construct and cotransfected a number of transcription factors, including c-jun, c-fos, p65 of nuclear factor-κB, etc. They found that although some of these major transcription factors activated the PRODH promoter, their effect was modest (<2-fold). Surprisingly, peroxisomal PPARγ was a potent activator. Transient transfection with PPARγ, together with troglitazone treatment, increased reporter expression >10-fold (30). We were interested in this finding because the pharmacologic ligands of PPARγ, the thiazolidinediones (TZD) have been used widely as oral hypoglycemic agents for patients with type 2 diabetes mellitus (31). Additionally, TZD can retard atherosclerotic processes (32). But most importantly, they have antiproliferative and apoptotic effects on a variety of cultured cancer cells and animal tumor models, leading to the proposal that TZD can be used for cancer prevention and/or treatment (33–35). Because the pharmacologic ligands of PPARγ was prostaglandin J2 (36), investigators proposed that PPARγ responds to inflammatory stimuli (prostaglandin J2) and may be a mechanism to halt chronic inflammation (37). Thus, PPARγ signaling and the response of POX/PRODH may be considered a response to inflammatory stress.

Treatment of a variety of cells with TZD markedly increased POX/PRODH activity and protein (29); the mechanism of this effect was transcriptional, as shown by activation of the promoter and by the binding of PPARγ to the peroxisomal proliferator response element from the PRODH promoter. The latter was shown by electrophoretic mobility shift assays and by chromosomal immunoprecipitation assays. These findings conclusively demonstrated that POX/PRODH is upregulated by PPARγ and its ligands, the TZD, leading us to conclude that POX/PRODH increases in response to inflammatory stress.

Because POX/PRODH is upregulated directly by both genotoxic and inflammatory stress, we considered whether it would also be responsive to nutrient stress. This stress paradigm plays an important role in cancer, because tumor cells detached from the basement membrane are isolated from their blood supply (38). The resultant hypoxic stress and nutrient stress activate several important responses for survival. Neangiogenesis stimulated by the transcription factor, hypoxia-inducible factor-1α, is the response to hypoxia; the oxygen-dependent...
prolylhydroxylation of hypoxia-inducible factor-1α and its von-Hippel-Lindau-directed proteasomal degradation has been characterized (39). For nutrient stress, a constellation of signaling pathways and responses has been described and the central mediator of these responses has been shown to be the serine-threonine kinase, mammalian target of rapamycin (mTOR) (40,41).

mTOR integrates metabolic information from several sources to regulate cell behavior (40,41), including growth factor signaling, amino acid availability, and adequate bioenergetics. A serine-threonine kinase, mTOR phosphorylates initiation factors and ribosomal proteins to activate protein translation and cell proliferation, respectively. Using RKO colorectal cancer cells, we showed that with decreasing glucose concentrations in the medium, the phosphorylation of mTOR decreased and the phosphorylation of S6K was proportionately decreased (J. Pandhare, J. M. Phang, unpublished data). On the other hand, the activity of POX/PRODH was markedly increased. That this response was mTOR mediated was shown with rapamycin, the specific inhibitor of mTOR signaling. At 10 nM, the phosphorylation of mTOR was markedly decreased and the phosphorylation of S6K was essentially abolished. Interestingly, rapamycin markedly upregulated POX/PRODH, plateauing at a level >10-fold higher than controls (J. Pandhare, J. M. Phang, unpublished data). The details of these studies are being published elsewhere. Thus, a regulatory pathway central to nutrient stress is a robust upregulator of POX/PRODH.

To summarize up to this point, POX/PRODH, the rate-limiting enzyme in proline degradation, was shown to be upregulated under 3 stress situations, thereby satisfying criterion 1. It was induced by p53, the primary mechanism for signaling genotoxic stress; by PPARγ and its pharmacologic ligands, a signaling system responding to inflammatory stress; and by modulating mTOR, the major signaling mechanism for nutrient stress, either directly with rapamycin or indirectly by 5-aminoimidazole-4-carboxamide-1β-D-ribonucleoside, the activator of AMP-directed protein kinase, a sensing mechanism for cellular bioenergetics.

Reservoir of mobilizable proline

Although POX/PRODH, the enzyme machinery for utilizing proline, is markedly upregulated under stress conditions, the important question remains as to the source of this proline (criterion 2). Because dietary proline is plentiful and proline biosynthesis is ubiquitous, it is unlikely that these sources would be regulated by stress. Although glutamine is the most abundant free amino acid in the body, its delivery requires an intact circulatory system and it is constantly biosynthesized and degraded as part of interorgan transfer (20,21). Total body glycine (free and protein bound) is also abundant, but it is rapidly metabolized into a number of products and the conversions are not amenable to specific regulation (42). The metabolism of proline, on the other hand, is regulated by several stress responses and it is abundant in collagen. Together with hydroxyproline, proline constitutes over 25% of collagen amino acids (43). Collagen is 80% of extracellular matrix (ECM) and 90–95% of the connective tissue and the organic part of bone (43). In fact, collagen makes up 25% of total body protein (44). A crude calculation based on 11.0 kg of total body protein in a 70-kg man (45) results in an estimate of 0.7 kg dry weight of proline/hydroxyproline in the body. The degradation of collagen is catalyzed by MMP (46,47), a family of metalloenzymes induced under conditions paralleling the stress conditions in which POX is induced, i.e. with genotoxic stress (46), inflammatory stress (47), and nutrient stress (48,49) (Fig. 3). Observing that MMP are activated during tumor invasion and metastasis (50), investigators proposed that pharmacologic blockade of MMP would be a useful approach for cancer chemotherapy (18). With encouraging results from preclinical studies (17), clinical trials using broad spectrum inhibitors of MMP were initiated, but the results were generally disappointing (18). At present, this area is being revisited, because the degradation of ECM by MMP can yield a variety of bioactive factors either as proteolytic products or as defined growth factors bound to ECM (51). These are interesting possibilities and deserve additional studies. However, the metabolic consequences of ECM degradation with the microenvironmental release of proline and hydroxyproline has not been considered.

We propose that altered proline/hydroxyproline metabolism is an important endpoint of MMP activation. A decrease in the effects of proline/hydroxyproline metabolism with pharmacologic blockade of MMP may contribute to the observed anticancer effects, but these may be offset by sources of these stress substrates during clinical trials, e.g. from an ad libitum diet.

If the stress-dependent activation of MMP results in the availability of proline and hydroxyproline, there should be observable evidence of collagen degradation, which is indeed the case. Fries et al. (52) showed that rats treated with trinitrobenzenesulphonic acid to induce colitis by inflammatory stress increased their urinary excretion of hydroxyproline normalized to creatinine >2-fold. Under these conditions, urinary hydroxyproline is an indication of collagen degradation. In similar studies, Reddy and Dhar (53) showed that Freund’s adjuvant-induced arthritis in rats was accompanied by increased collagen metabolism as indicated by a 2-fold increase in urinary hydroxyproline. The most relevant study was by Marian and Mazzucco (54) in the skin tumorigenesis model in mice. In this model, shaved skin is painted first with a carcinogen, e.g. benzo(a) pyrene or dimethylbenzanthrene, followed by repeated paintings with a tumor promoter, 12-O-tetradecanoylphorbol-13-acetate. After 5 paintings (2 wk) with 12-O-tetradecanoylphorbol-13-acetate, dermal collagen as measured by hydroxyproline content had decreased by >20%. Because collagen synthesis was increased as measured by an independent methodology, the decrease in net collagen content must reflect markedly increased collagen degradation (54). Thus, collagen degradation is a notable corollary of skin tumorigenesis. Although this phenomenon has not been emphasized in the carcinogenic process, these...
findings show that a variety of stress responses is accompanied by collagen degradation.

In our formulation of proline metabolism as a stress substrate, we have shown that the enzyme catalyzing the degradation of proline is upregulated by p53 (27,28), PPARγ (30), and rapamycin (J. Pandhare, J. M. Phang, unpublished data). Furthermore, the literature provided examples by which some of these responses activated the degradation of collagen (55,56), which would produce an increase in availability of proline as a stress substrate.

**Special functions of proline metabolism**

The final criterion for proline as a stress substrate is whether its metabolism serves special functions for the cell. As we previously mentioned, Donald et al. (28) showed that cytotoxic drugs increased POX/PRODH expression only with functioning p53 and that overexpression of POX/PRODH resulted in proline-dependent generation of ROS. Andy Hu et al. (29), using the DLD-1-tet-off- POX cells, showed that overexpression of enzymatically active POX/PRODH induced proline-dependent apoptosis, which was inhibited by treatment with antioxidants. Steve Maxwell et al. at Texas A & M University suggested that the mechanism of the apoptotic effect was due to P5C. However, unhydrolyzed P5C-dinitrophenylhydrazone from a commercial source was added directly to the incubation medium as the source of P5C (57). In a subsequent study, Maxwell et al. (58) also showed that the effect of POX/PRODH expression was mediated by ROS. They made an important additional discovery: POX/PRODH expression induced the calcineurin-dependent expression of nuclear factor of activated T cells, which played a role in apoptosis.

The mechanism of proline-dependent apoptosis was shown by Liu et al. (8). Using hydroethidine as a specific fluorescent indicator for superoxide, they showed that superoxide was generated in cells overexpressing POX/PRODH. Additionally, by coexpressing several antioxidant enzymes, i.e. SOD1, SOD2, and catalase, they showed that apoptosis was inhibited by the coexpression of SOD2 (MnSOD) but not by SOD1 (CuZnSOD) or catalase. This was not surprising, because MnSOD localizes to mitochondria and the finding supports the interpretation that the apoptotic effect of POX/PRODH is causally related to the proline-dependent generation of mitochondrial superoxide. The following sequence of events occurs. Superoxide alters mitochondrial membrane potential, allowing for the release of cytochrome c into the cytosol, followed by the activation of caspase 9 and the caspase cascade. Downstream events include cell cycle arrest, cleavage of poly (ADP ribose) polymerase, and DNA fragmentation. Not only the intrinsic (mitochondrial) apoptotic pathway but also the extrinsic (death receptor) pathway was activated by POX/PRODH (59). The mechanism for this latter pathway appears to be the upregulation of nuclear factor of activated T cells (58,59), which is a potent activator of the tumor necrosis factor-related apoptosis-inducing ligand promoter (59), the signaling of which leads to the activation of the caspase 8 limb of the caspase cascade (effects shown schematically in Fig. 4).

The aforementioned induction of POX/PRODH by PPARγ and its pharmacologic ligands, the TZD, was of special interest, because these agents are potent inhibitors of cancer cell growth (33–35). It has been proposed that this regulator of metabolism may be a promising target for the treatment of cancer (60). A variety of cultured cancer cells will undergo apoptosis when treated by TZD. Obviously, the induction of POX/PRODH by TZD suggested that POX/PRODH may be involved. In fact, the generation of ROS with TZD treatment was markedly inhibited by knockdown of POX/PRODH by its small interfering RNA (30). Our findings using colorectal cancer cells were corroborated by studies performed by others using non-small-cell lung cancer cells (61). Thus, it appears that the anticancer effects of PPARγ and TZD may be critically dependent upon their induction of POX/PRODH. Certainly, the effects on superoxide and apoptosis, and linkage with PPARγ are special functions of proline as a stress substrate (Fig. 4).

It is tempting to speculate that POX/PRODH is intrinsically able to perform the aforementioned task. Although it can contribute proline-derived electrons to the electron transport chain for use in reduction of oxygen to form superoxide, it would have no advantage over other sources of substrate such as succinate or NADH. The work from Tanner’s laboratory has shed light on this process. Using the monofunctional POX/PRODH from *Thermus thermophilus*, White et al. (9) showed that the FAD contained in the active site is exposed to solvent oxygen. The recombinant enzyme can indeed generate superoxide. If the mammalian enzyme is structurally similar, White’s finding suggests that POX/PRODH may be a controlled metabolic source of superoxide as an oxidizing signal. Although the pool of ROS may be generated by the “leakage of electrons,” it is rapidly detoxified by scavenging mechanisms and the complement of antioxidant enzymes (62). The proline-derived superoxide may be part of a special controlled mechanism for apoptotic signaling.

Another special function of proline as stress substrate is related to the induction of POX/PRODH by rapamycin. As described above, treatment of RKO colorectal cancer cells with Rapamycin upregulated POX/PRODH activity >10-fold (J. Pandhare, J. M. Phang, unpublished data). Rapamycin, by inhibiting mTOR, decreases protein synthesis and cell proliferation, switching the cell to a catabolic, survival mode. The maintenance of cellular bioenergetics is a critical mechanism for survival. Importantly, rapamycin treatment maintained ATP levels for at least 24 h (42) Using dehydroproline, an inhibitor of POX/PRODH catalytic activity, we showed that the maintenance of ATP levels in the presence or absence of added medium proline was markedly inhibited (63). Thus, the upregulation of

**FIGURE 4** POX produces superoxide that activates both limbs of the apoptotic pathway. Abbreviations: TRAIL, tumor necrosis factor related apoptosis-inducing ligand; DR5, death receptor 5, PARP, polyadenoribosyl polymerase; MEK, MAP kinase kinase; ERK, extracellular-signal regulated MAP kinase.
POX/PRODH by rapamycin and the contribution of POX/PRODH to the maintenance of ATP suggest that proline also can contribute to the bioenergetics needed for survival.

Although the metabolism of proline sequentially yields P5C, glutamate, and α-ketoglutarate, and thus can play an anaplerotic role for the TCA cycle (1,3), this is not the only source of bioenergetic maintenance activated by proline and POX/PRODH. The proline cycle and its metabolic interlock with the pentose phosphate pathway provide another mechanism (3,5,11). The significance of this interlock is to provide an alternative pathway for metabolizing glucose to generate reduced pyridine nucleotide (NADPH), which is shuttled into mitochondria by the cycling of proline (Fig. 2). To determine whether such a metabolic interlock is operative, we measured the conversion of $1^{-13}$C-glucose to $1^{13}$CO$_2$ and found that the expression of POX in the DLD-tet-off POX cells increased the pentose phosphate pathway $>5$-fold (63). By contrast, glycolysis as measured by the production of $\text{H}_2\text{O}$ from 5-$^3$H-glucose was only modestly increased (25%). Thus, the activation of POX augments bioenergetics not only by supplying carbons to the TCA cycle but also by the recruitment of the pentose phosphate pathway linked by the proline cycle to supplement bioenergetics (Fig. 2).

These 2 special functions of proline as stress substrate (criterion 3), i.e. maintenance of bioenergetics for survival vs. generation of superoxide as an oxidizing signal for programmed cell death, introduce an apparent paradox. Here, the structural biology model again provides an important insight. As previously described, the production of superoxide by the recombinant, purified protein was demonstrated in vitro (9). Furthermore, crystallographic studies identified an adjacent α helix shielding the FAD from solvent oxygen so that the enzyme has a switch, which directs electrons from proline either into the electron transport chain for the generation of ATP or allows exposure to solvent oxygen to generate superoxide (9). Whether this model also applies to the human enzyme has yet to be shown and the mechanism(s) controlling this switch will be of great interest.

Because the focus of the 7th Amino Acid Assessment Workshop and this special issue is on supplementation of amino acids, a few words here on proline supplementation would be appropriate. Our emphasis is on the mobilization of proline from endogenous stores (ECM) and its utilization. These processes are markedly upregulated by stress signaling. There is no evidence that dietary supplementation of proline modulates this signaling. However, the question of augmentation by supplementation under stress conditions has not been directly addressed experimentally. Under normal conditions, the circulating level of the proline degradative product, P5C, is affected by intake of food, but the relationship to a specific nutrient could not be shown (64). On the other hand, when blockade of MMP is considered as a therapeutic regimen for cancer, the limitation of the supply of proline from collagen may be a contributing mechanism. Thus, the potential antitumor effect of MMP blockade may require the limitation of alternative sources of proline/hydroxyproline, i.e. from the diet.

In summary, we propose that proline is a special microenvironmental stress substrate and we have provided evidence satisfying the 3 important criteria for such a substrate: 1) the enzyme utilizing proline, POX/PRODH, is responsive to genotoxic, inflammatory, and to nutrient stresses; 2) there is a large reservoir of proline in collagen and MMP are activated under conditions in which stress signals are activated (evidence from previously published reports shows degradation of collagen with the release of peptides accompanies these stress conditions); and 3) the degradation of proline serves the special functions of programmed cell death and augmentation of bioenergetics to maintain survival. Evidence from structural biology provides a potential “switching” mechanism intrinsic to POX/PRODH to mediate alternatively both these processes.

Other articles in this supplement include references (65–74).

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Literature Cited


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