Citrullination: Taking the Charge out of Arg

Protein citrullination (a.k.a. deimination) is a novel arginine-directed post-translational modification (PTM) that results in a permanent change in the targeted protein. Peptidylarginine deiminases (PADs) mediate the calcium-dependent deimination of the guanidino group of arginine side chains to form an ureido group and the nonstandard amino acid citrulline (see Fig. 1). There are 5 different PAD isoforms (PAD1-4, PAD6) that share significant sequence homology and differ primarily in their tissue-specific expression1. PADs are incapable of deiminating free L-arginine, which confirms their primary role in the modification of arginine side chains present in proteins. To date, there have been no enzymes identified that can reverse this process.

The deimination of arginine side chains in proteins results in the net loss of a positive charge and an increase in local hydrophobicity for the target protein. The biochemical implications of protein citrullination include protein unfolding2, loss of protein:protein interactions and/or interactions with other cellular components3, interference with other signaling events (e.g., arginine methylation4,5), and the unveiling of novel antigenic epitopes that can elicit immune responses and autoimmunity6.

Although the consequences of citrullination appear to negatively impact protein function, it is important to realize that this is a physiologically important process. Citrullinated proteins play essential roles in differentiation, nerve growth, embryonic development, cell death, and gene regulation6. Some biologically-relevant proteins known to be citrullinated by PADs include keratin, filaggrin, trichohyalin, vimentin, myelin basic protein (MBP), histones, α-enolase, fibrinogen, fibrils, collagen type I and II, β-actin, and tubulin5,11. It is noteworthy that several of these proteins are part of the cytoskeleton and/or are structural in nature.

Pathological protein citrullination has been associated with a range of diseases including multiple sclerosis, Alzheimer’s disease, rheumatoid arthritis (RA), psoriasis, prion disease, liver fibrosis, chronic obstructive pulmonary disease (COPD), and cancers12,13. The fact that most, if not all, of these diseases have an inflammatory component to their pathology is consistent with the importance of PADs in inflammation14. In the case of RA, several proteins have been identified that are specifically citrullinated in the synovial fluid of arthritic joints15; many of which are mentioned above. The citrullination of these proteins results in novel epitopes that give rise to autoantibodies16, and the resulting anti-citrullinated protein antibodies (ACPAs) have become a standard diagnostic and prognostic indicator for RA16-17. Circulating ACPAs are often present before other symptoms of RA and they are associated with an earlier onset of the disease, more severe joint damage, and a higher risk of cardiovascular comorbidities15-17.

![Figure 1. Citrullination of peptidyl-arginine by peptidylarginine deiminases (PADs).](image-url)
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lymphocytes\(^2\). Substantial immune cell apoptosis occurs in the synovial fluid of RA patients and further research is needed to understand if apoptosis is the primary mechanism by which the normally intracellular vimentin becomes extracellular and is able to elicit an autoimmune response.

Importantly, first and second generation PAD inhibitors have shown promise in preclinical studies with animal models of diseases where protein citrullination is known to be important\(^3\). It will be exciting to witness the maturation of PAD inhibitors over the next several years and see the development of inhibitors that have the potency, selectivity, and pharmacological properties needed to progress into human clinical trials.

### References


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