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Coenzyme, Cofactor and Prosthetic Group — Ambiguous Biochemical Jargon

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Introduction

For enzymes which require non-protein components to be functionally active, it is generally accepted that the term *holoenzyme* refers to the complete catalytic entity which consists of a protein part known as the *apoenzyme* and a non-protein constituent. The terms used for the functionally required non-protein constituents however, are ambiguous and differ from one commonly used biochemistry textbook to another. In certain textbooks, any associative non-protein factor required for the enzymatic function is termed *coenzyme*. The *coenzyme* may either be a *prosthetic group* — the tightly bound coenzyme, or a *cofactor* — the loosely bound small organic or inorganic molecule.

> *The cofactors are those small organic or inorganic molecules that the enzyme requires for activity . . . The prosthetic group is similar to the cofactor but is tightly bound to the apoenzyme,. Devlin*[1]

> *Most coenzymes are linked to enzymes by noncovalent forces. Those which form covalent bonds to enzymes may also be termed prosthetic groups.* Murray *et al*[2]

> *A coenzyme may be defined as a molecule that possesses physicochemical properties not found in the polypeptide chain of the enzyme and that acts together with the enzyme to catalyze a biochemical reaction . . . Tightly bound coenzymes are sometimes referred to as prosthetic groups.* Zubay[3]

Controversial Terminology

Whilst it is generally clear that the *prosthetic group* is a strongly bonded non-polypeptide helper component of a *holoenzyme* (as well as a non-catalytic protein) as described in most major biochemistry textbooks, the question of whether it could also be termed a *coenzyme* or a *cofactor* is a matter for debate. Textbooks by Lehninger *et al*[4] and Voet and Voet[5] describe the *cofactor* as the universal enzyme-associated non-protein factor which may either be a *prosthetic group* — a metal ion essentially permanently associated with its protein, or a *coenzyme* — a small organic molecule transiently associated with enzyme. However, unlike Voet and Voet, who classify *coenzyme* and *prosthetic group* separately, Lehninger *et al* further asserts that a *coenzyme* that is strongly bound to the enzyme is also considered a *prosthetic group*.

> *The cofactor may be either one or more inorganic ions . . . or complex organic or metalloorganic molecule called a coenzyme . . . A coenzyme or metal ion that is covalently bound to the enzyme protein is called a prosthetic group.* Lehninger *et al*[4]

> *Cofactors may be metal ions . . . or organic molecules known as coenzymes . . . Some cofactors . . . are but transiently associated with a given enzyme molecule . . . Other cofactors, known as prosthetic groups, are essentially permanently associated with their protein . . . Voet and Voet*[5]

In most textbooks, the *coenzyme* is also said to be chemically changed by enzymatic reaction. Regeneration of the molecule may be catalysed by a different enzyme. As such, the *coenzyme* essentially functions as a co-substrate. Conforming to this latter description of *coenzyme*, Montgomery *et al*[6] further defined the terms *coenzyme* and *cofactor* according to their roles in catalytic reaction. Whilst *coenzyme* is described as an organic

molecule directly involved in catalysis, a *cofactor* (organic molecule or metal ion) does not directly participate in the catalytic event.

> *Small organic molecules . . . essential to maintain certain enzyme proteins in a conformation suitable for catalysis . . . are regarded as cofactors even though they do not directly participate in the catalytic event . . . The term coenzyme applies to organic molecules . . . which are essential for activity of numerous enzymes.* Montgomery *et al*[6]

Contrary to the initial terminology however, a *cofactor* in this case is not necessarily by definition a *coenzyme*. To avoid controversy, it is apparent that several textbooks of biochemistry have uncannily refrained using the term *cofactor*. Textbooks by Stryer,[7] Murray *et al*[2] and Zubay[3] for instance, considered any associative enzymatic non-protein factor as *coenzyme*, with no mention of *cofactor*.

The Cause

Perhaps one of the contributing factors that lead to the confusion in terminology is the lack of elaborative definition of terms. The terms, *cofactor*, *coenzyme* and *prosthetic group* have been defined through different perspectives. A *prosthetic group* is generally defined based on its covalent association with enzyme or proteins. *Cofactor* and *coenzyme* however, are considered through their roles in enzyme catalysed reaction (or protein function). Due to this reason, any restrictive measure confining certain substances strictly to certain terms would only result in controversial and antagonistic terminology.

Prosthetic group, *coenzyme* and *cofactor* are all generally defined. Whilst a *cofactor* is any factor essentially required for enzyme activity or protein function, a *coenzyme* is the *cofactor* which is directly involved in enzyme catalysed reaction. A *cofactor* which is not directly involved in enzyme catalysis, or is associated with the function of a non-catalytic protein is not a *coenzyme*. A *prosthetic group* (covalently associated non-protein constituent required for a particular function), on the other hand, may also be termed a *coenzyme* if it is directly involved in catalytic reaction. A *prosthetic group* which is not involved in enzyme catalysed reaction but functionally essential to the enzyme or a non-catalytic protein is also called a *cofactor*. *Coenzyme* and *cofactor* weakly bound to enzyme or protein are however, not classified as *prosthetic group*.

The Solution

As teachers of biochemistry to science and medical undergraduates, we often encounter questions pertaining to these controversial terms. To avoid confusion and permanently resolve the prevalent controversy, we strongly feel that more emphasis and elaboration should be presented in defining each of the terms in all textbooks of biochemistry.

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Book Review

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Methods in Molecular Biology, Volume 27: Biomembrane Protocols: II. Architecture and Function

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The structures of biological membranes and the various functions that are carried out by membrane systems are areas of research which are essential to the work not only of biochemists and cell biologists but also immunologists, parasitologists, virologists and molecular biologists. Thus numerous books are currently available, and no doubt many more will appear over the coming years, which attempt to cover some of the techniques currently used to explore this important and multidisciplinary area of research. *Biomembrane Protocols: II Architecture and Function* is a companion to an earlier volume in the Methods in Molecular Biology series *Biomembrane Protocols: I Isolation and Analysis*. This new volume is concerned exclusively with the organisation of membrane components and how this organisation controls function. Of the 24 chapters, the earlier ones cover topics such as crystallisation of membrane proteins for X-ray analysis, determination of cell surface polarity and topography, the use of antipeptide and monoclonal antibodies for the isolation and characterisation of membrane proteins, and the reconstitution of proteins into lipid vesicles. Several chapters then deal with a variety of techniques used to measure protein–protein and protein–lipid interactions, phospholipid topography, asymmetry and movement. The later chapters deal with more specific membrane functions such as G-protein analysis, assay of protein kinases, phosphoinositide analysis and calcium measurements. The last four chapters cover topics as diverse as membrane permeabilisation with bacterial toxins, measurement of ion fluxes and pH gradients across membranes, and ligand binding and processing. Each topic is briefly introduced and then the experimental protocols are presented in a step-by-step, easy to follow style. Numerous explanatory notes are included to help you avoid the not-so-obvious pitfalls and benefit from the authors' own experience. Thus, at least in theory, 'the reader will be able to perform the techniques without the need to consult other texts'. All in all, a useful book which covers a very wide range of topics used to study the structure and function of biological membranes.

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