One protein—Many functions

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The concept of one enzyme-one activity had influenced biochemistry for over half a century. Over 1000 enzymes are now described. Many of them are highly 'specific'. Some of them are crystallized and their three-dimensional structures determined. They range from 12 to 1000 kDa in molecular weight and possess 124 to several hundreds of amino acids. They occur as single polypeptides or multiple-subunit proteins. The active sites are assembled on these by appropriate tertiary folding of the polypeptide chain, or by interaction of the constituent subunits. The substrate is held by the side-chains of a few amino acids at the active site on the surface, occupying a tiny fraction of the total area. What is the bulk of the protein behind the active site doing? Do all proteins have only one function each? Why not a protein have more than one active site on its large surface? Will we discover more than one activity for some proteins? These newer possibilities are emerging and are finding experimental support. Some proteins purified to homogeneity using assay methods for different activities are now recognized to have the same molecular weight and a high degree of homology of amino acid sequence. Obviously they are identical. They represent the phenomenon of one protein—many functions.

A review on proteins with more than one activity, called multifunctional, was available in 1976 (ref. 1). Some examples are described for a single protein with multiple catalytic sites in distinct domains. In other words, a protein can be visualized as a collection of domains on the surface fused together! A protein may be associated with other proteins(s) in hetero-oligomeric structure, or may be embedded in a membrane and exhibit another activity. The number of such proteins is increasing and some of these are described here (Table 1).

Protein disulphide isomerase (PDI)

This enzyme, discovered by Anfinsen and coworkers[2] in liver microsomes, catalyses the rearrangement of disulphide bonds during protein folding. Given the name protein disulphide isomerase, it has 'unimpressive' kcat value (0.4/min) but occurs ubiquitously in mammalian tissues at high concentration. It is a homodimer of 58-kDa protein located on the lumen side of endoplasmic reticulum[3]. Each monomer has two identical, independently active sites with a sequence—Try.Cys.Gly.His.Cys.Lys (WCGHCK), first Cys being essential. This sequence is similar to that of thioredoxin and β-subunit of follicle stimulating and lutenizing hormones. PDI has thioredoxin-like activity and it can act as a substrate for thioredoxin reductase[4].

PDI has the ability to bind to peptides of various lengths and sequences with little specificity. These peptides act as competitive inhibitors of its isomerase activity[5]. The protein-folding activity was shown to be inhibited by Cd^{++} and arsenite, both specific dithiol-complexing compounds[6], possibly by blocking the two cysteines in the thioredoxin-like active sites. The protein-binding activity in the cadmium-treated protein was found to remain unaffected[7]. The two actions are therefore carried out by two distinct domains of the protein. In an yeast mutant, a truncated PDI protein, without a third of its C-terminal portion, possessed the isomerase activity but the peptide-binding activity and the viability of the cells decreased[8]. It appears that the peptide-binding activity of PDI is more important in the cell. Does this protein have other more relevant functions?

Although PDI is ubiquitous, it occurs with high specific activities (concentrations?) in collagen-synthesizing cells, and interestingly correlates with prolyl-4-hydroxylase (P4H). The β-subunit of P4H has 94% homology with PDI[9]. Could this then be the primary function of this protein wrongly identified as PDI? P4H is a 240 kDa-protein with two 64 kDa α-subunits and two β-subunits of PDI. In the complex the PDI expresses only half its isomerase activity.

A thyroxine-binding protein (K_D for T_3 57 nM)[10, 11] and another protein with thyroxine-5′-deiodinase activity[12] in liver microsomes were found to be 58 kDa-proteins. In both cases the amino acid sequences of the respective purified proteins were independently determined and were found to be identical with PDI.

The binding-protein of the glycosylation site, Asn.aa.Ser/Thr, a component of oligosaccharyl transferase was found to be same as PDI[13]. But it was not involved in the N-glycosylation process. However, its apparent molecular weight increased to 60 kDa by glycosylation[14].

Being a component of microsomal triglyceride-transfer protein (MTP), PDI plays a role in the exchange of triglyceride between membranes, and possibly plasma lipoproteins[15]. This protein, a heterodimer of a 58 kDa-protein (PDI) and a 88 kDa-protein, was isolated from lumen of microsomes of liver and intestines where such exchanges occur, but was not detected in brain, heart and kidney. Association of the two proteins drastically decreased the isomerase activity of PDI and increased the lipid-transfer activity. The 88 kDa-protein was not

Table 1. One protein—Many functions

Primary identity	Other activities
Protein disulfide isomerase 58 kDa	Thioredoxin-like activity Peptide-binding (sequence non-specific) Prolyl-4-hydroxylase- subunit Thyroxine-binding Oligosaccharyl transferase component Microsomal triglyceride transfer protein-subunit
58 kDa-cytosolic proteins (a) Pyruvate kinase (b) Non-specific lipid transfer protein (c) Fermodulin	 T_3-binding protein Sterol carrier protein 2 Fe-binding protein
Peptidyl-prolyl-*cis-trans* isomerase 17 kDa	Cyclophilin Cyclosporin A-binding
Glyceraldehyde-3-phosphate dehydrogenase 37 kDa	Acyl phosphatase Esterase ADP-ribosylation Microtubule bundling Protein-kinase Uracil-DNA glycosylase (nuclear) tRNA-binding (sequence specific) Amyloid protein (Amy C)-binding Membrane-binding
Lens crystallins: delta epsilon eta lambda mu rho tau sigma	 Arginine-succinate lyase Lactate dehydrogenase-B Aldehyde dehydrogenase Hydroxylacyl CoA dehydrogenase Ornithine cyclodeaminase NADPH-quinone reductase Enolase GSH-S-transferase
Aconitase (cytosolic with Fe-S cluster)	Iron-response element binding protein (Fe-free)
(a) Isocitrate dehydrogenase (NAD^+ specific) (b) Isocitrate dehydrogenase ($NADP^+$ specific) 40 kDa	Mitochondrial mRNA-binding Vanadate-dependent NADH oxidation
Lactoferrin (Fe-binding) 80 kDa	Ribonuclease
Heat shock proteins (hsp)	Peroxidase (Mn) Alpha-subunit of F_1-ATPase
(a) Oxygenase from *P. syringe* (b) Mitochondrial protease (c) Amylase/Trypsin inhibitor	Mono-oxygenase/dioxygenase reactions Non-overlapping specificities of peptidase action Independent inhibition of two activities
Leukocyte antigen CD 38 46 kDa	NAD glycohydrolase ADP-ribose cyclase Cyclic ADP-ribose hydrolase

detected in abetalipoproteinemia[16].

PDI was found to be the major auto-immune antigenic protein in LEC strain of rats prone to spontaneous, hereditary hepatitis[17].

The various functions of this 58 kDa-protein are schematically shown in Figure 1.

Pyruvate kinase

An extranuclear tri-iodothyronine (T_3)-binding protein was identified as a 58 kDa-protein in the cytosol possessing an amino acid sequence entirely different from PDI[18]. This sequence was found to be homologous to the subunit of pyruvate kinase subtype M_2, found in many tissues, with its amounts increasing in proliferating liver tissue. The purified monomeric 58 kDa-protein showed only about 5% of pyruvate kinase activity . It is converted to the tetramer with full activity in the presence of fructose-1, 6-bisphosphate. Binding of thyroid hormones to the tetrameric protein dissociated it to the monomeric form with loss of the kinase activity. T_3 was most efficient with a $K_i = 30$ nM. Both forms of the protein may thus exist simultaneously depending on the concentrations of the two ligands.

Some examples are given below: delta in reptiles—arginosuccinte lyase; epsilon in birds—lactate dehydrogenase-B; zeta in camel—NADPH-quinone oxidoreductase; eta in elephants—aldehyde dehydrogenase; lambda in rabbit—hydroxyacyl CoA dehydrogenase; mu in kangaroo—ornithine cyclodeaminase; rho in frogs—NADPH-acceptor reductase; tau in turtle—enolase; sigma in cephalopods—GSH-S-transferase. These proteins are recruited from the available enzyme-proteins in the cell and then structured into the transparent lens. It is amazing how such bulk protein is accumulated at the site and folded differently from its native enzyme structure apparently by some guided process.

Aconitase sans Fe-S cluster

Aconitase catalyses the interconversion of citrate and isocitrate, involving a dehydration-hydration reaction. This non-oxidative enzyme has an Fe-S cluster with labile sulphide and is also identified as the high potential iron-sulphur protein (HiPIP). Full activity of this enzyme needed addition of iron in the assay, known to convert [3Fe-4S] to-[4Fe-4S] cubane cluster.

The most obvious function of aconitase is in citric cycle and therefore it is in mitochondria. But a cytosolic form of the enzyme is known for a long time which is distinct and coded by a gene on a different chromosome. The importance of this protein in the cytosol became apparent when it was found to have high degree of homology with the binding protein of iron-responsive elements (IRE). These are present in the messenger RNAs of transferrin receptor and of ferritin in the form of RNA stem-loop structures located in the untranslated regions. The iron-sensing is carried out by the protein IRE-BP which on binding to IRE with high affinity 'exerts an iron-dependent, dual, and reciprocal control of transferrin receptors and ferritin concentrations'. The cytosolic aconitase without the Fe-S clusters is identical with IRE-BP, and the two forms of the protein coexist[33].

Isocitrate dehydrogenase

The NAD^+-dependent isocitrate dehydrogenase in yeast mitochondria and a 40 kDa-protein that binds to the 5′-untranslated leaders of mitochondrial mRNA are the same[34].

The $NADP^+$-specific isocitrate dehydrogenase protein was able to oxidize NADH, but not NADPH, in presence of polyvanadate[35].

Human lactoferrin

Human milk was found to have potent ribonuclease activity which turned out to be 80 kDa-Fe-binding glycoprotein, an isoform of lactoferrin sharing physical, chemical and antigenic properties but differing from it in the lack of significant iron-binding capacity[36].

ATPase and stress-induced peroxidase

F_1-ATPase in mammalian mitochondria has multiple subunits of which the α-subunit is considered regulatory and the β-subunit binds to nucleotides. The α-subunit, a 57 kDa-protein, was also found to occur in peroxisomes, and to respond to heat shock. It belongs to the stress proteins of hsp-60 family[37].

A manganese-dependent peroxidase induced in *Neurospora crassa* by H_2O_2, ethanol, arsenite or $CdCl_2$ was identified as a heat shock protein[38].

Single enzyme catalysing two independent reactions

It is possible that two genes are 'fused' and give a single mRNA translated into the product as a fusion protein having two independent activities. Two subunits having different activities may thus form a single protein. Some examples are available in this category.

Two reactions were found to be catalysed by a single enzyme isolated from *Pseudomonas syringe*. Acting on 2-oxoglutarate in the presence of arginine, the products produced by mono-oxygenase reaction were succinate, hydroxy arginine and CO_2, and that by dioxygenase were ethylene and $3CO_2$ (ref. 39).

Maturation of imported proteins into mitochondria is carried out by signal peptidases. A mitochondrial protease with 'two catalytic subunits of non-overlapping specificities' was recently described[40].

On the same basis as activities, multifunctional or multiheaded inhibitors representing a sum of individual capabilities are also known to act on more than one enzyme with separate non-overlapping binding site for each[41–43]. A ragi amylase/trypsin inhibitor (RATI), stable to exposure to 8M urea or heating at 90°, inhibiting amylase and trypsin activities[43] is an example.

Human leukocyte antigen CD 38

The 46 kDa-protein on the outer surface of human erythrocytes identified immunologically as the antigen CD 38 was found to have three related NAD^+-metabolizing enzymes—NAD^+ glycohydrolase, ADP-ribose cyclase and cyclic ADP-ribose hydrolase[44, 45]—involved in the formation and degradation of cyclic ADP-ribose, emerging as an important calcium-mobilizing molecule. This glycoprotein, bound to the membrane by a transmembrane domain and a short cytoplasmic tail, acts as a surface

antigen and is involved in activation and proliferation of lymphocytes. A vignette of a general phenomenon of two related activities resident in a protein is obvious.

Perspective remarks

The number of proteins present in any cell is limited whereas the functions performed by them are many. About fifty bands are visualized on SDS-PAGE analysis of proteins of tissues. About 20 of these are major and some may occur in too small concentrations to be detected. At the best the total number of individual proteins may not far exceed 100. With some of these, the cell has obviously devised ways of structuring them to multiple active forms.

The proteins are large and their surfaces can provide multiple active sites of different catalytic potential. The examples available already indicate specific, individually active domains fused together into a protein showing multifaceted activities. With minor chemical modifications of the amino acids some proteins gain or lose activities that can be used also as part of metabolic regulation. Binding of effector molecules alter the subunit structures and show totally different activities. By co-operative hetero-oligomer formation between two or more proteins, an entirely new activity can emerge. Embedding in the membranes gives the proteins additional potential for altered forms and activities. Indeed the process of guided folding, now an active field of research, can provide multiple stable structures that can coexist with multiplicity of activities. This is exemplified by the phenomenon of one mutation and multiple syndromes[46].

Three distinct ways of using a protein become apparent from the examples available: multiple catalytic sites, binding properties and structural roles. Thus, a protein active as an enzyme can be 'recruited' to do other functions. It can bind to its own substrate or other metabolites (e.g. tRNA) and transport them. Complexing with distinct structures in mRNA or DNA, it can play a regulatory role. The regulatory elements and transcription factors may turn out to be known enzyme proteins. It can become a structural element like in lens crystallins or in the subunit or membrane structures. Indeed, the possibilities are legion.

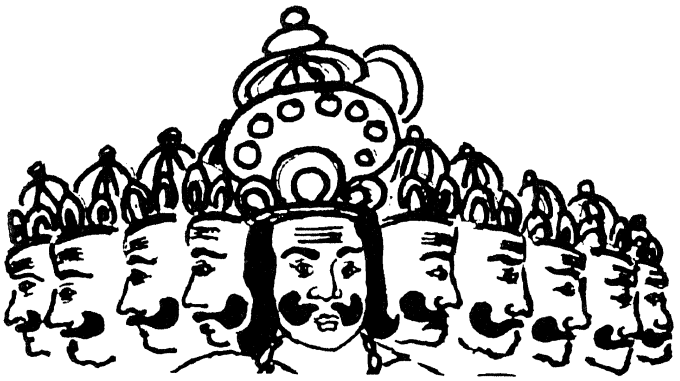
One other perspective is emerging. How valid is the enzyme nomenclature in the light of these developments? In the foregoing examples, it can be seen that a 58 kDa-protein is identified with at least four distinct catalytic activities, and there are atleast two distinctive 58 kDa-proteins with other activities. It is probably time to consider developing a 'protein nomenclature' based on domain-level amino acid sequence, structural motifs and their arrangements in polypeptides. More and more proteins will now be identified with multiple functions and the author will be grateful if the readers draw the attention to other examples.

1. Kirschner, K. and Bisswanger, H., *Annu. Rev. Biochem.*, 1976, **45**, 143–166.
2. Goldberger, R. F., Epstein, C. J. and Anfinsen, C. B., *J. Biol. Chem.*, 1963, **238**, 628–635.
3. Freedman, R. B., Bulleid, N. J., Hawkins, H. C. and Paver, P. L., *Biochem. Soc. Symp.*, 1989, **55**, 167–192.
4. Lundstrom, J. and Holmgren, A., *J. Biol. Chem.*, 1990, **265**, 9114–9120.
5. Noiva, R., Kimura, H., Roos, J. and Lennarz, W. J., *J. Biol. Chem.*, 1991, **266**, 19645–19649.
6. Ramakrishna Kurup, C. K., Raman, T. S. and Ramasarma, T., *Biochem. Biophys. Acta*, 1966, **113**, 255–276.
7. Noiva, R., Freedman, R. B. and Lennarz, W. J., *J. Biol. Chem.*, 1993, **268**, 19210–19217.
8. LaMantia, M. and Lennarz, W. J., *Cell*, 1993, **74**, 899–908.
9. Philajaniemi, T., Helaakoski, J., Tasanen, K., Myllyla, R., Huhtala, M. L., Kova, J. and Kivlrikko, K., *EMBO J.*, 1987, **6**, 443–449.
10. Yamauchi, K., Yamamato, T., Hayashi, H., Koya, S., Takikawa, H., Toyoshima, K. and Horiuchi, R., *Biochem. Biophys. Res. Commun.*, 1987, **146**, 1485–1492.
11. Cheng, S., Gong, Q., Parkinson, C., Robinson, C. A., Appella, F., Merlino, G. J. and Pastan, I., *J. Biol. Chem.*, 1987, **262**, 11221–11227.
12. Boada, R. J., Campbell, D. A. and Chopra, I., *Biochem. Biophys. Res. Commun.*, 1988, **155**, 1297–1304.
13. LaMantia, M. L., Muira, T., Tachikawa, H., Kaplan, H. A., Lennarz, W. J. and Mizemaga, T., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 4453–4457.
14. Kaplan, H. A., Naider, F. and Lennarz, W. J., *J. Biol. Chem.*, 1988, **263**, 7814–7820.
15. Wetterau, J. R., Combs, K. A., Spinner, S. N. and Joiner, B. J., *J. Biol. Chem.*, 1990, **265**, 9800–9807.
16. Sharp, D. and Wetterau, J. R., *Nature*, 1993, **365**, 65–69.
17. Yokoi, T., Nagayama, S., Kajwara, R., Kawaguchi, Y., Honuchi, R. and Kamataki, T., *Biochem. Biophys. Acta*, 1993, **1158**, 339–344.
18. Kato, H., Fukuda, T., Parkinson, C., McPhie, P. and Cheng, S. Y., *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 7861–7865.
19. Ossendorp, B. C., Van Heusden, G. P., de Beer, A. L. J., Bos, K., Schouten, G. L. and Wirtz, K. W. A. *Eur. J. Biochem.*, 1991, **201**, 233–239.
20. Satish Menon, A., Usha Devi, S. and Ramasarma, T., *Arch. Biochem. Biophys.*, 1985, **239**, 342–351.
21. Takahashi, N., Hayano, T. and Suzuki, K., *Nature*, 1989, **337**, 473–475.
22. Fischer, G., Wittman-Liebold, E., Lang, K., Kiefhaba, T. and Schmid, F. X., *Nature*, 1989, **337**, 476–478.
23. Harris, J. I. and Waters, M., *The Enzymes*, 1976, **13**, 1–49.
24. Kots, A. Y., Skurat, A. V., Sergienko, E. A., Bulargina, T. and Severin, E. S., *FEBS Lett.*, 1993, **300**, 9–12.
25. Vaidyanathan, V. V., Sastry, P. S. and Ramasarma, T., *Biochem. Biophys. Acta*, 1993, **1203**, 36–44.
26. Kots, A. Y., Sergienko, E. A, Bulargina, T. V. and Severin, E. A., *FEBS Lett.*, 1993, **324**, 33–36.
27. Huitorel, R. M. and Pantoloni, D., *Eur. J. Biochem.*, 1985, **150**, 265–269; *Biochemistry*, 1985, **25**, 626–661.
28. Kawamoto, R. M. and Calwell, A. K., *Biochemistry*, 1986, **25**, 626–661.
29. Meyer-Siegler, K., Mauro, D. J., Seal, G., Wirzer, J., DeRiel, J. K. and Sirover, M. A., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 840–846.
30. Singh, R. and Green, M. R., *Science*, 1993, **259**, 365–368.
31. Schulze. H., Schuler, A., Stuber, D., Dobeli, H., Langen, H. and

Huber, G., *J. Neurochem.*, 1993, **60**, 1915–1922.
32. Wistow, G. *Trends Biol. Sci.*, 1993, **18**, 301–306.
33. Beinert, H. and Kennedy, M. C., *FASEB J.*, 1993, **7**, 1442–1449.
34. Elzinga, S. D. J., Bednarz, A. L., Van Oostenum, K., Dekker, P. J. J. and Grivell, L. A., *Nucleic Acids Res.*, 1993, **21**, 5328–5331.
35. Kalyani, P., Gullapalli, S., Rau, M. and Ramasarma, T., *Mol. Cell. Biochem.*, 1991, **107**, 31–37.
36. Furmanski, P., Li, Z., Fortuna, M. B., Swamy, C. V. B. and Das, M. R., *J. Exp. Med.*, 1989, **170**, 415–429.
37. Luis, A. M., Alconada, A. and Cuezva, J. M., *J. Biol. Chem.*, 1990, **265**, 7713–7716.
38. Machwe, A. and Kapoor, M., *Biochem. Biophys. Res. Commun.*, 1993, **196**, 692–698.
39. Fukuda, H., Ozawa, T., Tazaki, M., Nagahama, K., Fujii, T., Tanase, S. and Morino, Y., *Biochem. Biophys. Res. Commun.*, 1992, **188**, 483–489.
40. Nunnari, J., Food, T. D. and Walter, P., *Science*, 1993, **262**, 1979–2004.
41. Mundy, J., Hejgaard, J. and Sevendsen, I., *FEBS Lett.*, 1984, **167**, 210–214.
42. Rhodes, M. B., Bennet, N. and Feeny, R. E., *J. Biol. Chem.*, 1960, **235**, 1689–1693.
43. Alagiri, S. and Singh, T. P., *Biochim. Biophys. Acta*, 1993, **1203**, 77–84.
44. Takasawa, S., Tohgo, A., Noguchi, N., Koguma, T., Nata, K., Sugimato, T., Yonekura, H. and Okamato, H., *J. Biol. Chem.*, 1993, **268**, 26052–26054.
45. Zoechi, E., Francis, L., Guide, L., Benatti, U., Bargellesi, A., Malavasi, F., Lee, H. C. and DeFlora, A., *Biochem. Biophys. Res. Commun.*, 1993, **196**, 1459–1465.
46. Van Heyninger, V., *Nature*, 1994, **367**, 319–321.

ACKNOWLEDGEMETS. The financial assistance by the Department of Science and Technology, Government of India is acknowledged. TR is an emeritus scientist of the Council of Scientific and Industrial Research, New Delhi.

Received 29 March 1994; accepted 4 April 1994



In the epic *Ramayana*, the king of Lanka, Ravana, depicted to have ten faces, is a great warrior, a scholar of scriptures and an accomplished veena player—a multifaceted personality! (idea conceived by V. V. Vaidyanathan and drawn by M. Anil Kumar).

Leaf gas exchange in lightflecks of plants of different successional range in the understorey of a Central European beech forest

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Young seedlings of two vines, in particular *Clematis vitalba* L. and *Rubus fructicosus* agg, and of seven other plants all co-occurring in a European beech forest, were subjected to lightflecks. We study their induction dynamics in particular, since induction state controls light use efficiency, light quantum yield and carbon gain. Distinct differences have been observed in the velocity of photosynthetic induction increase and loss. Pioneer species take a longer time to reach half induction in continuous saturating light as compared to late successional representatives. Despite the identical growth conditions, photosynthesis is induced faster in the pioneer species and the induction is lost slower. This is important for seedling establishment in the understorey.

THE light environment in forest understoreys typically consists of long periods of low PFD[1] separated by periods of short but frequent sunflecks lasting from less than a second up to a few seconds, and rarely sunflecks lasting up to several minutes[1-4]. In a variety of forests it has been shown that sunflecks contribute between 35% and 75% to total daily photon flux[1,3,5]. Current evidence suggests that a large portion of carbon gain is attributable to these higher light phases in the understorey.

For the first lightflecks after darkness or a prolonged dim light phase, photosynthetic carbon gain by a leaf is likely to be limited by low photosynthetic induction. This limitation, however, is gradually removed during subsequent lightflecks[6-8]. Understorey plant species accomplish a considerable portion of their daily carbon