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REVIEW

CONTROL AT THE LEVEL OF SUBSTRATE SUPPLY—AN ALTERNATIVE IN THE REGULATION OF PHENYLPROPANOID ACCUMULATION IN PLANT CELLS

U. MARGNA
Institute of Experimental Biology, 203051 Harku, Estonian S.S.R., U.S.S.R.

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Key Word Index—flavonoids; cinnamic acid derivatives; phenylpropanoids; phenylalanine; phenylalanine ammonia lyase (PAL); control of accumulation; regulation of synthesis.

Abstract—The problem of the intracellular mechanisms responsible for the control of accumulation of phenylalanine-derived polyphenols in plants is considered. Possible control functions of phenylalanine ammonia lyase (PAL) in this process are critically discussed and experimental facts are surveyed providing evidence that substrate (phenylalanine) supply rather than enzymic (PAL) activity is the most likely limiting factor in controlling phenylpropanoid accumulation. These facts involve: frequent lack of consistent correlation between changes in the level of PAL and accumulation rate of phenylpropanoids, high deaminating capacity of PAL markedly exceeding the level required to sustain the formation of polyphenols in most tissues, stimulatory action of phenylalanine on polyphenol accumulation when administered externally, very low and constant levels of free endogenous phenylalanine in plant tissues, and the existence of balanced relationship between protein metabolism and the formation of flavonoids and cinnamic acid derivatives in plants.

INTRODUCTION

The accumulation of flavonoids and other phenylalanine-derived polyphenols is generally considered as a process which is controlled at the enzymic level [1–8]. However, under physiological conditions normally present in a plant cell, the enzymic activity needs not to be always the main rate-limiting factor. Experimental data are available suggesting that the accumulation of phenylpropanoids may be also controlled by another cellular mechanism not directly related to the catalytic activity of the enzymes involved. This is the control at the level of substrate supply, an alternative so far practically overlooked. Hints about the possible limiting role of a substrate factor can be found in only a few reports [9–12].

The objective of this paper is to discuss this problem in more detail. It surveys the experimental evidence which does not match satisfactorily with the concept of enzymic limitations as the primary controlling factor of phenylpropanoid accumulation, but, in contrast, seems to support the idea of substrate regulation.

DISCUSSION

Phenylalanine deamination—the critical point of regulation

A control mechanism functioning on substrate supply seems to be operating at the first step of the biosynthetic pathway of cinnamic acids, flavonoids and other related compounds—at the point of phenylalanine deamination catalyzed by phenylalanine ammonia-lyase (PAL) (Fig. 1).

Since the discovery and the first characterization of PAL by Koukol and Conn 1961 [13], the deamination of phenylalanine has been extensively studied and much data on physiological and biochemical aspects of this reaction have accumulated. The avitivity of PAL is often correlated with simultaneous changes in the rate of accumulation of flavonoids and related phenolics. This correlation suggests a possible causal relationship between the two biochemical parameters and it is widely believed, therefore, that PAL is the main limiting factor in the biosynethsis of flavonoids, cinnamic acids and other phenylpropanoids [14, 15].

In spite of the convincing correlative evidence, there is the objection that the correlation, though recorded

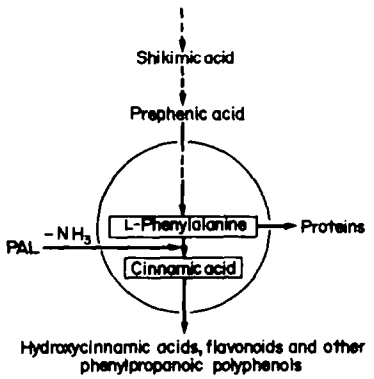

Fig. 1. PAL-mediated deamination of phenylalanine into *trans*-cinnamic acid—the initial step in the biosynthetic pathway of hydroxycinnamic acids, flavonoids and other phenylpropanoids.

frequently, is still not absolute. Thus, in strawberry leaf disks floated in water, flavonoid formation shows clear phytochrome reversibility whereas there are no changes in the activity of PAL associated with short exposures to red or far-red light [16]. Gherkin seedlings held at 10° develop high PAL levels even in the dark, but the increase in PAL activity is accompanied by an increase in the accumulation of hydroxycinnamic acids only when the plants are exposed to light [17]. In the hooks and cotyledons of radish seedlings irradiated with far-red, anthocyanins accumulate when PAL activity is already declining [18]. A marked discrepancy in timing between the maximum rate of polyphenol accumulation and the period of maximum PAL activity was observed also in rose cell suspensions cultured in the presence of 2,4-D[19], in developing *Narcissus* flowers [20] and in etiolated shoots of oat seedlings [8]. In carrot tissue cultures, gibberellic acid strongly inhibits the accumulation of anthocyanins, but shows no effect upon the level of PAL activity [21]. By contrast, another growth hormone, kinetin, stimulates the formation of anthocyanins in dark-grown red cabbage seedlings, but, again, fails to have any effect upon the enzyme [22]. Administration of certain DNA intercalating compounds into excised pea pods leads to increased production of pisatin, an isoflavonoid phytoalexin, but with no concomitant increase in PAL. Several other compounds of this kind have just the opposite effect, i.e. give rise to an increase in the levels of PAL activity without any parallel changes in the formation of the isoflavonoid [23]. Similar lack of consistent correlation between phenylpropanoid accumulation and PAL activity was osberved in pea pods treated with several drugs [24], several homopolymeric peptides in combination with other inducers [25], and in a number of other cases [10, 12, 26–29].

Still more intriguing, however, are the observations that the accumulation rate of phenolics may increase while PAL activity is decreasing [30, 31]. Thus, administration of phenylalanine into seedling tissues can bring about a considerable increase in the accumulation of anthocyanins in almost all cases while PAL activity at the same time exhibits a decrease (Fig. 2). A series of feeding experiments with excised buckwheat hypocotyls and cotyledons has shown that this negative correlation is equally valid with respect to the leucoanthocyanidins [32, 33], rutin and glycoflavones (unpublished results of this laboratory), and chlorogenic acid [34], all of which, contrary to the responses observed in the acitivity of PAL [30], exhibited a more or less distinct increase in their quantity under these experimental conditions. Exogenous supply with phenylalanine stimulates also the accumulation of chlorogenic acid in potato tuber slices [35] and cinnamic acids in strawberry leaf disks [12] while in both tissues the treatment, again, results in a decrease in PAL activity.

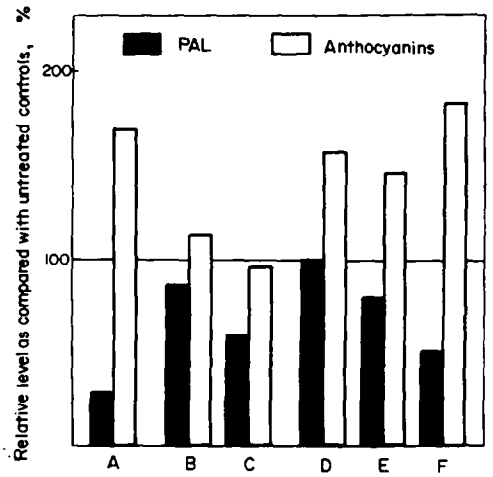

Fig. 2. Influence of phenylalanine on the activity of PAL and accumulation of anthocyanins in several plant seedlings (in % units as compared with the level of PAL and antocyanins in the untreated material). A—hypocotyls of radish seedlings (intact); B—primary leaf of rye seedlings (intact); C—excised primary leaf of rye seedlings; D—hypocotyls of buckwheat seedlings (intact); E—excised buckwheat hypocotyls; F—excised buckwheat cotyledons.

Similar results have been recorded under a variety of experimental conditions. Sweetclover leaves being developed after the plants have been defoliated contain sharply increased levels of *o*-hydroxycinnamic acid, although the activity of PAL, in general, is considerably reduced [26]. In cell cultures of *Haplopappus gracilis* grown in a medium with 10^{-6} M α-naphthaleneacetic acid, PAL activity, after reaching a maximum at 4–8 hr after inoculation, decreases first rapidly and then slowly until the end of the 6-day experiment. This decrease, however, does not hinder progressive increase in the content of anthocyanins during the same period [36]. Dark-grown rose cell suspension cultures supplemented with actinomycin-D and cycloheximide show an immediate and rapid decline in PAL activity, but the overall rise in the content of polyphenols is only slightly reduced or, after later addition of antibiotics, even stimulated [19]. PAL and related polyphenols may exhibit opposite responses under the influence of different temperatures. Thus, within physiological limits a rise in growing temperature results in a decrease in the activity of PAL in buckwheat seedlings, but the accumulation of flavonoids is generally enhanced; conversely, lowering the temperature increases PAL activity but decreases flavonoid concentration [37, 38].

Although changes in the level of a single or a group of related derivatives need not necessarily coincide with the changes taking place in the level of all polyphenols, it is still possible that the total (unknown) production of phenylpropanoid compounds in all these cases is still positively correlated with changes in PAL activity.

May PAL really be a limiting factor?

In a search for the answer to this question, one should, first of all, consider the catalytic potential of PAL in plants. Swain and Williams [39] were the first to stress this aspect in their studies on flavonoid biosynthesis. They found, by reexamining the data produced in five different laboratories, that the potential capacity of PAL to deaminate phenylalanine tended to be much higher (in 3 cases of the 5) than that which could have been expected from the amount of phenolic compounds actually synthesized. Moreover, in all the three cases giving high calculated values for PAL activity, there was sufficient enzyme in the dark-grown tissue to account for the increase in the rate of phenolic synthesis in the light. This finding is in contrast to the widespread opinion that the light-induced changes in the accumulation of phenolics may be determined by the primary events at the level of PAL [14].

The lack of quantitative correspondence between

changes in the activity of PAL and the rate of synthesis of phenolic compounds is open to at least two possible explanations. Swain and Williams [39] interpreted it as casting some doubt on the role of phenylalanine as an obligate precursor of cinnamic acids and flavonoids in all plants (see also [27, 40–43]). An alternative, however, is that PAL represents one of the (constitutive?) enzymes whose catalytic power remains sufficient under all circumstances. In that case a change in PAL activity need not be followed by an adequate reflection at the level of subsequent reactions.

Little information is available at present to verify this assumption yet the calculations of several authors (besides Swain and Williams [39] already cited) are all in favour of this view. Thus, Maier and Hasegawa [44] have found that in developing grapefruit a linear relationship exists between PAL activity and the rate of naringenin glycoside accumulation. The relationship, with the molar ratio of *in vitro* PAL activity to the rate of *in situ* naringenin glycoside accumulation of 3.19 holds over a range in which the extreme rates of flavanone accumulation vary a 100-fold. They viewed this molar ratio as an evidence that grapefruits have potential PAL activity sufficient to account for the observed rate of naringenin glycoside accumulation in the intact fruit.

Ahmed and Swain [45] have found that the deaminating capacity of PAL at the level of activity observed in dark-grown mung bean seedlings (69 nmol of cinnamic acid/min/g fr. wt) was in about 2.5-fold excess over the amount of soluble phenylpropanoid (mainly chlorogenic acid) synthesized in light-treated tissue (28 nmol/min/g fr. wt). Since the total activity of PAL approximately doubled in the light-treated material, the ratio of PAL activity to the rate of phenylpropanoid accumulation of 5:1 was actually characteristic of this tissue. The same ratio of PAL activity to the rate of accumulation of flavonols has been observed also in isolated onion scales [46]. The calculations made by Chulia and Tissut [20] in their studies with developing *Narcissus* flowers showed that at the observed level of PAL activity the capacity of flower tissues to produce cinnamic acid moieties was 2.5–7 times (in some stages even up to 180 times) higher than the average daily increments in flavonol synthesis. In another investigation of the same authors [47] the quantitative relation between the highest values of PAL activity and the intensity of flavonol accumulation was found to be 1.1:1 in developing *Forsythia* flowers and 123:1 in young maize leaves.

Table 1. Catalytic activity of PAL* and flavonoid accumulation in plant tissues during a 40 hr experimental period (16 hr light + 24 hr darkness)

Plant tissue†	PAL, μmol/ seedling	Flavonoids‡ μmol/ seedling	Approximate ratio
Buckwheat seedlings (intact):			
hypocotyls	~1·0	0.099	10:1
cotyledons	~3.0	0.482	6:1
First primary leaf of rye seedlings:			
intact	0.752	0.0084	90:1
excised	0.724	0.0053	137:1
Hypocotyls of radish seedlings:			
intact	0.079	0.0061	13:1
excised	0.321	Traces	∞

* Expressed as the amount of *trans*-cinnamic acid which might have been produced theoretically during the experimental period at the level of PAL activity observed. † All seedlings grown in water; intact seedlings exposed to the light at the age of 56 hr, excised organs—at the age of 80 hr. ‡ In buckwheat seedlings—total sum of anthocyanins, leucoanthocyanidins, rutin and glycoflavones (present in cotyledons only), in rye and radish seedlings—anthocyanins (the major flavonoid present).

The results of our own studies carried out with buckwheat, radish and rye seedlings [30, 31] lend further support to this idea. In all tissues studied (Table 1) the total catalytic potential of PAL was quite sufficient to deaminate much more phenylalanine than the amount actually required for the formation of the flavonoids. In the hypocotyls of dark-grown radish seedlings the catalytic power of PAL was somewhat smaller than that found for the seedlings illuminated, yet it was still enough to allow about 5 times more anthocyanin formation than that normally observed in the light-treated seedlings. In the first primary leaf of the etiolated rye seedlings the corresponding ratio was approximately 60:1. In coleoptiles of rye seedlings, the level of the PAL activity proved to be too low to permit accurate measurements yet the calculations showed that the synthesis of flavonoids with the rate typical of this tissue could be readily achieved even with the activity of PAL five times less than the minimal level of activity still detectable by the method used [31, 35].

Taking these findings into consideration, a broader reexamination of the PAL literature seemed to be necessary in order to establish whether an excessively high potential for deaminating phenylalanine (with respect to the actual extent or PAL-dependent polyphenol synthesis) is characteristic of only a limited number of plant species, or whether it is a phenomenon of more general importance. For this purpose, the total amount of *trans*-cinnamic acid was calculated that the plant tissues investigated would have been able to produce within a 24-hr time period at the level of PAL activity recorded. The estimates thus found for the deaminating capacity of tissues were then compared with the amount of phenylalanine-derived polyphenols accumulating in the same material and approximate ratios of the two values were calculated. The results of this comparison are noteworthy (Table 2). In 15 cases out of 20, the average or, in several tissues, even the lowest level of PAL activity was found to be more than sufficient to contribute within a one-day period many times more intense production of deaminated phenylalanine than that which would have been required for the biosynthesis of polyphenols at the maximal level of their accumulation observed. In parsley cell suspension cultues [55], etiolated buckwheat hypocotyls [56], spindle-tree leaves [57], and Satsuma mandarin fruit [61] the molar ratio of possible one-day production of cinnamic acid to the amount of polyphenols accumulated was somewhat lesser and varied within the limits of 0.7–1.3, while only in dark-grown rose cell suspension cultures [19] the relative deaminating capacity remained comparatively low and did not exceed the level required to sustain the formation of polyphenols.

Of course, total production of phenylalanine-derived phenolics was not measured in these studies and the ratios found only approximately characterize the relations between PAL activity and polyphenol accumulation. It

Table 2. Phenylalanine deaminating capacity of plant tissues as compared with the level of polyphenol accumulation observed

Tissue	Unit	PAL activity*, μmol/hr/unit	Deaminating capacity μmol/24/hr/unit (DC)	Polyphenols measured	Maximum content of polyphenols accumulated, μmol/unit (P)	Ratio DC:P	References
1	2	3	4	5	6	7	8
Potato tuber slices cultured in the light	g fr. wt	1–2	24–48	Chlorogenic acid	0.75	32–64	[35]
Mustard seedlings	seedling	0.0018	0.043	anthocyanins	0.019†	2.3	[48–50]
Gherkin seedling hypocotyls	hypocotyl	0.005	0.12	hydroxycinnamic acids	0.025	4.8	[1, 2]
Carrot root aggregens:	g fr. wt			anthocyanins,			[10]
type AGI		0.13	3.12	hydroxycinnamic	1.83‡	1.7	
type AGID		0.24	5.76	acids	0.45‡	12.8	
Sorghum shoots	g fr. wt	10	240	hydroxycinnamic acids, luteolin, dhurrin	10	24	[51]
Bean leaves:							
(a) healthy	g fr. wt	12.5	300	total polyphenols	3.20	94	[52]
(b) infected with tobacco necrosis virus							
2 days after infection		95.0	2280		4.16	548	
3 days after infection		132.1	3179		4.62	686	
Pea terminal buds	g fr. wt	5	120	flavonoids	13.0	9.2	[53, 54]
Root tips of maize seedlings:	10^6 cells			total polyphenols			[11]
dividing cells		5	120		0.2	600	
elongating cells		10.5	252		0.75	336	
mature cells		19	456		1.4	326	
Coleoptiles of maize seedlings:	10^6 cells			total polyphenols			[11]
dividing cells		3	72		1.25	58	
elongating cells		9	216		3.85	56	
mature cells		13	312		2.45	127	
Parsley cell suspension cultures grown under continuous illumination	g fr. wt	0.12	2.88	flavones	2.3	1.3	[55]
Buckwheat seedling hypocotyls, etiolated	hypocotyl	0.018	0.432	flavonoids, chlorogenic acid	0.445	1.0	[56]
Rose cell suspension cultures, dark-brown, cultured at 2,4-D levels:	culture			total polyphenols			[19]
5.10^{-7} M		2	48		330	0.15	
5.10^{-6} M		1	24		180	0.13	
1.10^{-4} M		0.7	16.8		90	0.19	
Spindle-tree (*Euonymus*) leaves, plants grown under:	cm^2			flavonoids,			[57]
inductive temperature conditions		0.0147	0.353	hydroxycinnamic acids	0.278	1.3	
noninductive temperature conditions		0.0047	0.113		0.170	0.7	
Strawberry leaves, plants grown at	cm^2			flavonoids,			[57]
10°		0.066	1.584	hydroxycinnamic	0.340	4.7	
25°		0.022	0.528	acids	0.172	3.1	
Evening-primrose (*Oenothera*) seedlings grown in continuous far-red light	seedling	0.0017	0.041	anthocyanins	0.001†	41	[58]
Orange flavedo callus tissue	g fr. wt	0.06	1.44	flavonoids	0.016‡	90	[59]
Poplar cell suspension culture	g fr. wt	1	24	anthocyanins	0.5	48	[60]
Satsuma mandarin fruit	g fr. wt	8	192	hesperidin	164	1.2	[61]
Kidney bean hypocotyls, excised	g fr. wt	0.186	4.46	flavonoids	0.174	26	[62]
Primary leaf of oat seedlings	leaf	0.06	1.44	glycoflavones	0.250	5.8	[8]

* In potato tuber tissues, parsley and rose cell suspension cultures, Satsuma mandarin fruit, and oat primary leaf—approximate average level over the whole experimental period followed; in mustard and buckwheat seedlings, and orange flavedo callus tissue—average dark level; in gherkin seedlings, pea terminal buds, and poplar cell suspensioncultures—the lowest dark level; in evening primrose seedlings—average over 9 plasmotypes; in sorghum shoots and kidney bean hypocotyls—the lowest single measurement result given; in other tissues—single measurement data given in the original tables or graphs. † Calculated from absorbance data; in the case of evening primrose seedlings average over 9 plasmotypes. ‡ Calculated from dry weight data assuming a mean 10% content of dry matter in fresh tissue.

must be emphasized, however, that the ratios have been calculated under the most severe conditions for PAL, i.e. possible *one-day production* of cinnamate at the *lowest level* (see legend to Table 2) of PAL activity reported has been compared with the *maximal amount* of phenolics accumulated by the tissue during the *whole experimental period*. Hence, the actual ratios 'total capacity of producing cinnamate/production of phenolics' should be, in fact, many times higher in these tissues than those presented in Table 2. It seems therefore that if the total amount of phenylalanine-derived compounds (including lignin precursors) even remained considerably underestimated in the ratio calculations made here and some turnover simultaneously occurred, the total cinnamate producing capacity of these tissues was still high enough to allow an unlimited synthesis of phenolics.

When attempting to calculate from the values given in Table 2, the possible percentage of cinnamic acid produced by PAL in the total amount of dry matter of the corresponding tissue (assuming a mean 10% content of dry matter in a fresh tissue), additional evidence for the high catalytic power of PAL can be obtained. Provided that the deaminating capacity of the enzyme is realized completely and *trans*-cinnamic acid formed is accumulating unaltered, the total amount of the cinnamate produced within a 24-hr period would, in a number of tissues, contribute a considerable part of the overall production of organic matter. Its share in dry weight would range from about 4% in the case of poplar cell suspension cultures [60] to about 4–7% in the case of potato tuber tissues [35], 18% in pea terminal buds [53, 54], 30% in Satsuma mandarin fruit [61], 36% in young sorghum shoots [51], and 45% in bean leaves [52], respectively. Of the same order of magnitude would be the calculated % values for the possible production of cinnamic acid also in *Xanthium* leaves [63], barley shoots and stems, and in alfalfa stems [13], while in bean leaves infected with tobacco necrosis virus [52] and in a variety of plant tissues (15 species) assayed for PAL by Jangaard [64] the reported levels of PAL activity would render possible the production of cinnamic acid in amounts which would account for the whole dry matter of the tissues within several hours. Since such enormous production of cinnamate cannot occur in reality, it is evident that in many plant tissues the natural catalytic efficiency of PAL can never be realized to its full extent.

If these ratio and per cent calculations are reliable, the following conclusion ensues: many if not all plant species appear to have acquired during their evolutionary development an enormous capacity to deaminate phenylalanine which many times exceeds the level theoretically required for an adequate supply of the phenylpropanoids it needs.

Phenylalanine—the limiting substrate

In the light of these arguments, it seems unlikely that any changes in the activity of PAL actually influence the rate of accumulation of flavonoids, cinnamic acid derivatives and other phenylalanine-derived polyphenols in plants. It is probable instead that the deaminating capacity of plant tissues is always sufficient to consume all the phenylalanine left over from protein synthesis that becomes available to the enzymic action of PAL during normal cell metabolism. Hence it follows that if there is a quantitative shift in the accumulation of flavonoids and related compounds, it can hardly be a result of the controlling action of PAL or, in more general terms, that produced by an alteration in the catalytic power of the enzymic involved. It is much more likely due to a change in the supply of primary substrate, i.e. phenylalanine, to the enzyme (see also [12]).

The simplest evidence for the limiting role of phenylalanine is its stimulatory action on polyphenol accumulation when the amino acid is administered externally. Exogenous supply with phenylalanine has been shown to give rise to an enhanced formation of anthocyanins in a variety of Cruciferae [31, 65, 66] and in rye seedlings [31], all kinds of flavonoids in buckwheat seedlings ([33, 66, 67]; and unpublished results of this laboratory), chlorogenic acid in buckwheat seedlings [34] and potato tuber tissue [35], cinnamic acids and flavolans in strawberry leaf disks [12], lignin-type polyphenols in the first internodes of *Sorghum* [68], and in a number of other cases [4, 69–71]. Although phenylalanine administered exogenously may not mix with the ednogenous pool of this amino acid in plant cells and the two pools may be metabolized independently, these data do support the idea that the supply of phenylalanine is normally rate-limiting. PAL activity tends to decrease under these feeding conditions [12, 30, 31, 35].

The same point is also supported by the data on the level of free phenylalanine in plant tissues. A survey of the literature shows that the content of free unbound phenylalanine is characteristically very low in plants and does not generally exceed 0.1–0.2 micromol/g fresh weight in a wide range of very different plant tissues [4, 65, 72–76]. Obviously there are many such tissues in which the content of free phenylalanine, in fact, does not reach that average level [65, 76–79] or even remains too low to allow accurate quantitative measurements [65, 80]. When roughly comparing these data with the estimates found by different invetsigators for PAL activity (Table 2), one can easily see that in most tissues the whole pool of free phenylalanine even when totally available to PAL action, would be completely exhausted within several minutes. This situation is not consistent with the possible control function on PAL postulated: the rate-limiting role of an enzyme requires that its primary substrate at least in several stages should be accumulating over the catalytic capacity actually present. Khavkin and Prelyayeva [11] have compared the content of free phenylalanine in maize seedlings with the kinetic properties of the purified PAL from the same source and have shown that at the K_m values 0.2–0.7 mM found [81] the supply of phenylalanine rather than the level of PAL activity must limit phenylpropanoid biosynthesis.

It is also significant that under normal physiological conditions the level of free phenylalanine is not only relatively low in plant tissues, but also tends to remain practically constant [4, 72, 73, 75, 76, 79, 82–84]. Mild stress factors such as mineral deficiency or abundant supply with mineral nutrients do not effect phenylalanine levels [76, 83]. Neither is this constancy changed in tissues encouraged to accumulate phenolics or incubated under conditions where a rapid synthesis of phenolic compounds is taking place [4, 84]. When phenylalanine is supplied externally a temporary rise in the level of cellular free phenylalanine occurs but as soon as the uptake is stopped the content of free phenylalanine in tissues rapidly levels down to its initial constant value [75]. These data appear to indicate that an active pool of free endogenous phenylalanine, in fact, is normally

absent from plant cells. The small amounts of free cellular phenylalanine may be interpreted merely as a certain minimal level of this amino acid unavailable for metabolic centres, above which every additional particle obviously, if it cannot be used for protein synthesis, undergoes an immediate deamination. This exaplanation is in a good agreement with the high catalytic potential of PAL observed in plants.

It must be noted here that under the influence of more powerful stress factors, i.e. high doses of gamma-irradiation and pathogen attacks leading to severe disturbances of metabolic activities, a considerable increase in the content of free phenylalanine may still occur in plants [72, 77, 85, 86]. Since increased phenolic biosynthesis is a typical response of plant tissues to both irradiation and infection, reflecting a stress condition [52, 77, 85, 87–89], a causal relationship between these two phenomena seems to be beyond doubt. Here the endogenous supply of phenylalanine plays a role in controlling the accumulation of phenolics.

There is still another set of data indicating that a balanced relationship between protein metabolism and the formation of flavonoids and cinnamic acid derivatives exists in plants. The relationship is typically manifested in the following manner: under conditions favourable for protein biosynthesis a decrease in the accumulation of phenylpropanoids generally occurs, while any conditions leading to a suppression of protein synthesis or favouring an acceleration of turnover and catabolic degradation of proteins have a stimulatory influence on this process. This kind of relationship exists, in principle, in every case where there occurs an environmental change leading to a shift in the metabolic reactions in plant cells [66, 90, 91] (see Table 3). It is obvious that protein biosynthesis as the process of paramount importance is the preferential consumer of phenylalanine produced by cell. The portion of phenylalanine becoming available for the formation of polyphenols is determined, therefore, by the extent of its utilization in the biosynthesis of new proteins, on the one hand, and the intensity of its release from protein molecules during their turnover, on the other (Fig. 3). The synchronized relationship between protein metabolism and polyphenol accumulation seem to prove unequivocally that this portion of phenylalanine is normally far less than the catalytic capacity of PAL and never reaches the level completely saturating the enzymic apparatus involved in the biosynthesis of cinnamic acids, flavonoids and other related polyphenols.

Table 3. Balanced relationship between protein metabolism (A) and phenylpropanoid accumulation (B) in plants

Conditions	A	B	References
Influence of ionizing radiation	−	+	[77, 87, 89, 92, 93]
Pathogen attack	−	+	[52, 85, 94]
Mineral deficiency	−	+	[95–99]
Water stress	−	+	[95, 100, 101]
Senescence of tissues	−	+	[92, 95, 102–104]
Influence of antibiotics	−	+	[105–109]
Abundant nitrogen fertilization	+	−	[97, 110–113]
Administration of nitrogen into tissues	+	−	[66, 99, 107, 114–118]
Systematic infection with mosaic viruses	+	−	[119, 120]

− Suppression; + Stimulation

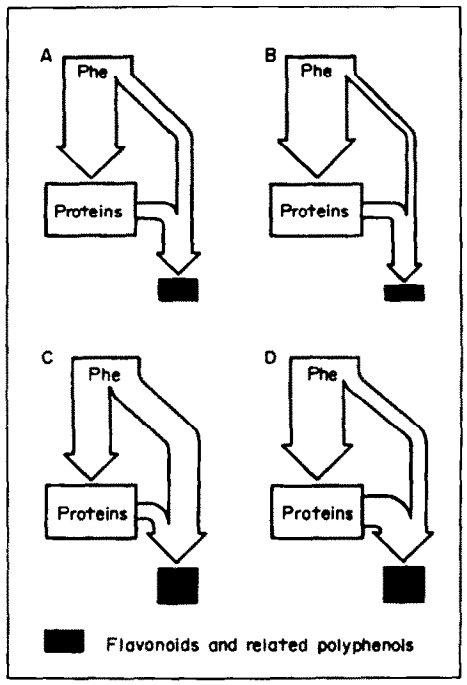

Fig. 3. Balanced relationship between protein metabolism and phenylpropanoid accumulation as a reflection of changes in the distribution of phenylananine between proteins and polyphenols. A—initial state; B—protein biosynthesis enhanced; C—protein biosynthesis suppressed; D—protein turnover accelerated. Phe—phenylalanine.

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