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Review

The role of emerging techniques in the investigation of prolidase deficiency: From diagnosis to the development of a possible therapeutical approach

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Abstract

The aim of the present article is to review the efforts performed in the past two decades by numerous research groups for the development of methods that allow a correct diagnosis of prolidase deficiency (PD), a rare autosomal recessive disorder and for the rationalization of a possible therapeutic intervention on these patients. In particular, the interest of the reader is focused on the application of capillary electrophoresis (i) for the detection of biological markers that reflect the pathological feature of the disease and (ii) for the determination of the efficiency of a carrier system in delivering prolidase inside cells in a possible therapy based on enzyme replacement.
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Keywords: Prolidase deficiency; Capillary electrophoresis; Liposomes; Diagnosis; Urine; Imidodipeptides

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1. Introduction

Prolidase deficiency (PD) is a severe autosomal recessive disorder, chronic in nature, progressive and debilitating due to the lack of prolidase (EC 3.4.13.9), a peptidase with a preference for X_{aa}-Pro dipeptide substrates that participates in collagen metabolism and in the terminal degradation of endogenous and dietary proteins [1–9]. Being a very rare disorder according to the classification of the European Community (that considers a rare pathology to be one suffered by less than 5:10,000 inhabitants of the Union) [10], PD falls into the "orphan" disease category and, like most rare diseases, it has long been a mystery disorder

Abbreviations: PD, prolidase deficiency; CE, capillary electrophoresis
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[10,11]. It typically begins in childhood; common symptoms include chronic intractable skin ulcerations and mental retardation [9]. The condition generally progresses in adulthood to include splenomegaly and recurrent respiratory infections that may lead to end-stage widespread infections. Given that many of these symptoms are often mistaken for a host of other medical problems, people with PD often are misdiagnosed thus being subjected to a frustrating path of medical solutions. Their struggles in achieving a correct diagnosis then are met with the further challenges of managing a disease that has no treatment. In fact, being infrequent, PD attracts little, if any, interest from the pharmaceutical industry that is not involved in sponsoring trials to attempt to improve the outcome of this disease. Given that the therapeutic intervention for PD more often adopted to date (a mere management of ulcerations) does not address the basic cause of the disorder, this results in immense implications for patients who feel disenfranchised in terms of hoping for an effective outcome from their disease [12–17]. Thus, in view of the importance of a correct diagnosis, several research groups have worked on the improvement of methods to achieve the standards of reproducibility and performance that are expected of clinical tests. Likely, to provide a standardized platform for the rationalization of a possible therapeutic intervention, numerous attempts aimed at providing a concrete answer to these patients have been performed. In support to a review article concerning the list of clinical features of prolidase deficiency published a few years ago [18], the present report addresses the recent trends in diagnostic and therapeutic areas and provides examples of how new methodological procedures may give a significant contribution to improvements in prolidase deficiency investigation.

2. Methods available for diagnosing prolidase deficiency

To date, numerous different methods have been developed for diagnosing prolidase deficiency. They are mainly based (i) on the determination of enzyme activity in erythrocytes, leukocytes and/or skin fibroblast cultures of PD patients or (ii) on the screening of X-Pro imidodipeptides massively excreted in their urine.

The following paragraphs cover the literature of the past two decades and, also in the light of our own experience, critically discuss the results in terms of the analytical and clinical interest of these procedures.

2.1. Determination of prolidase activity

The oldest and most classic method of detection of prolidase activity relies on the colorimetric reaction of proline using Chinard's reagent following hydrolysis of X-Pro peptide substrate [19]. The liberated proline reacts with ninhydrin to form a red product that is read at 515 nm.

Chinard's original method, modified by Myara et al. [20] to increase its sensitivity, was later successfully applied to the determination of prolidase activity in eryhtrocyte haemolysates and dermal fibroblasts. Although still in use [21], this method is time-consuming and not very specific since the presence of other aminoacids (i.e. lysine and ornithine) and of the reduced form of glutathione can interfere with the colorimetric reaction. Conversely, the spectrophotometric method cannot be applied to substrates such as Phe-Pro because of interference by phenylalanine absorption [22]. The drawbacks mentioned above prompted studies to evaluate the feasibility of using other methods with greater specificity and sensitivity. In this context, Harada et al. [23] have developed a reverse-phase high-performance liquid chromatographic (RP-HPLC) procedure for determining prolidase activity. Following the disappearance of Gly-Pro peak (elution time around 5 min) upon incubation of substrate with pre-activated human serum from six healthy young adults, they have successfully applied their approach to the detection of prolidase activity in this matrix. Due to the low amount of sample required; the excellent resolution and short time needed for washing and re-equilibrating the column, capillary electrophoresis (CE) appeared to be even better suited than HPLC to monitoring small peptides such as the prolidase substrates X-Pro. This technique was developed almost 10 years ago in our laboratory and was applied to the determination of prolidase activity in fibroblast cultures from five controls and four PD patients [24]. Kinetic measurements of peptide bond hydrolysis were performed on different substrates by following the disappearance of the peptide's substrate peak and, in the case of Phe-Pro, simultaneous analysis of the product and parent peptide was possible. Comparison of the results with those obtained using the Chinard's colorimetric assay permitted us to establish that CE was superior to the former method in terms of accuracy, speed and specificity. The confirmation that CE was a viable analytical alternative to spectrophotometric approach was also obtained in our laboratory by applying successfully this procedure to the detection of prolidase activity in eryhtrocytes of a subject who presented with clinical symptoms (leg ulcerations) that had been mistaken for medical problems other than prolidase deficiency [25]. Our CE determinations allowed to achieve a correct diagnosis and to catalogue this as a very rare case of late onset of prolidase deficiency. The CE procedure was also very useful for determining the variation of prolidase activity levels in erythrocytes of two PD patients that had been submitted to a series of consecutive erythroexchange steps [26]. The kinetic of inactivation of fresh prolidase could also be determined. The high resolving capacity of CE allowed this to be considered as the only technique applicable for the determination of prolidase activity in complex matrices. In this context we have applied CE to detect prolidase levels in skin cultured fibroblasts from PD patients that had been incubated with prolidase-loaded liposomes [27]. The heavy interferences of liposomes with classical Chinard's assay completely prevented the use of the colorimetric procedure in medium, washings and cellular extracts. A series of CE runs performed using 50 mM sodium tetraborate, pH 9.3 with the addition of 30 mM α-cyclodextrin as background electrolyte allowed to measure the rate of disappearance of Gly-Pro dipeptide in the above matrices, thus confirming the extreme versatility of this technique. Detection and quantification of Gly-Pro dipeptide in medium, cell layer and matrix, obtained from long term cultured human fibroblasts of controls and PD patients, using the same CE approach have also been reported by other authors [28]. More recently also matrix-assisted laser

Table 1
Imidodipeptides identified in urine of PD patients; their molecular masses and relative abundance

Imidodipeptide	Molecular mass	Relative abundance
Ser-Pro	202	+
Asp-Pro	230	+
Gly-Pro	172	+++
Phe-Pro	262	+++
Phe-Hyp	278	+
Ala-Pro	186	+
Leu-Hyp	244	++
Pro-Pro	212	+
Leu-Pro	228	+++
Trp-Pro	301	+
His-Pro	252	+
Tyr-Pro	278	n.d.
Glu-Pro	244	n.d.
Asn-Pro	229	n.d.
Ile-Pro	228	n.d.
Thr-Pro	216	n.d.

Source: [30], n.d., not determined.

desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has been successfully applied to the determination of prolidase activity on serum of normal subjects and PD patients [29]. The activity of the enzyme was determined by following the complete disappearance of the Gly-Pro signal with the concomitant appearance of the Pro peak. Using this method the authors have unambiguously identified subjects with homozygous or heterozigous prolidase deficiency.

The variety of applications reported here shows that extremely powerful CE and MS systems have been designed as promising and reliable methods to assess prolidase activity. The fact that these techniques are not limited by drawbacks that may seriously affect the spectrophotometric determination make them very attractive and will certainly expand their role in this field.

2.2. *Screening of imidodipeptides*

A list of the most common imidodipeptides excreted in urine by patients with prolidase deficiency is reported in Table 1. In the seventies and early eighties, aminoacid analysis [31–34] and isotachophoresis [35] have been the methods of election to detect these compounds. However, as it has been previously indicated for the determination of prolidase activity, the need for accurate identification of minute amounts of these biomarkers present in a very complex mixture, as is urine, evidenced the serious drawbacks of the above techniques in relation to their poor resolving power and/or the long time required for sample pre-treatment. These obstacles have been partially overcome through the development of techniques with increased precision of analysis. The application of HPLC interfaced with atmospheric pressure ionization mass spectrometry (API-MS), for instance, allowed Kodama et al. [36] and Sugahara et al. [37] to observe and quantify in the urine of patients with prolidase deficiency the quasi-molecular ions $[M+H]^+$ of various imidodipeptides containing proline or hydroxyproline at the C-terminus. Although it was never used for diagnostic purposes on urine of PD patients, this method was further improved by Sugahara et al. [38] to measure simultaneously both imidodipeptides containing N- or C-terminal proline. The HPLC-API/MS method above indicated was applied by Sugahara et al. [39] also on serum of both normal subjects and PD patients. If the $[M+H]^+$ ions of imidodipeptides containing C-terminal proline were not observed in the sera of controls, they were identified in those of PD patients thus indicating that this could be a useful tool for the diagnosis of the disorder.

Given the encouraging results obtained upon application of CE to the determination of prolidase activity, we devoted our strenghts in developing a technique also for screening of urinary dipeptides. CE in fact is particularly attractive for the analysis of urine (and other biological fluids) because it very often allows the investigators to detect specific analytes without sample pre-treatment or clean up. A CE method was thus applied in our laboratory to investigate imidodipeptiduria in four PD patients [40]. The technique proved to be sensitive and fast with analysis times of less than 20 min being required for obtaining electrophoretic patterns in which the unique cluster of peaks representing imidodipeptides X-Pro/Hyp was reproducible for all patients. The efficiency of the method was even improved by separating the complex mixture of imidodipeptides with micellar electrokinetic chromatography (MEKC), in an effort to identify all electrophoretic peaks [41]. Taken together, these data lead us to propose CE as the method of choice for its routine use in clinical laboratories. The growing interest of patients' caregivers on this methodology allowed the Department of Biochemistry of the University of Pavia, to become the structure of reference in Italy for the investigation of this disease. Thus, although the rarity of the disorder makes it difficult to find on a regular basis sufficiently large cohorts of patients to improve understanding of and research into this disease, in few years we have been able to collect urine, serum and/or skin biopsies from 22 subjects, 12 males and 10 females. Sixteen of them were italian living in different regions and six were foreigners. All of them presented with clinical symptoms that could be related to PD, although no one had ever been diagnosed for this disorder. The availability of these specimens allowed us to carry on the largest CE screening ever performed before on individuals with these clinical features.

The results of systematic CE analysis showed that only the profiles of 10 urines (from four females and six males) were characterized by the presence of the huge number of overlapping peaks that we had previously identified as the typical mixture of imidodipeptides X-Pro excreted in urine by PD patients [40]. By contrast, the profiles of remaining 12 subjects (six males and six females) were very similar, if not totally identical, to those of healthy individuals. A few electropherograms from each group of subjects, chosen at random among those available and representative of all others, are shown in Fig. 1, panels A (traces b → e) and B (traces a → e), respectively. Based on our previous experience [38], only individuals belonging to the first group (10 subjects), indicated as $P_1 \rightarrow P_{10}$, were thus diagnosed as real PD patients. However, being our diagnostic approach based on the identification of a typical electrophoretic profile, obviously the

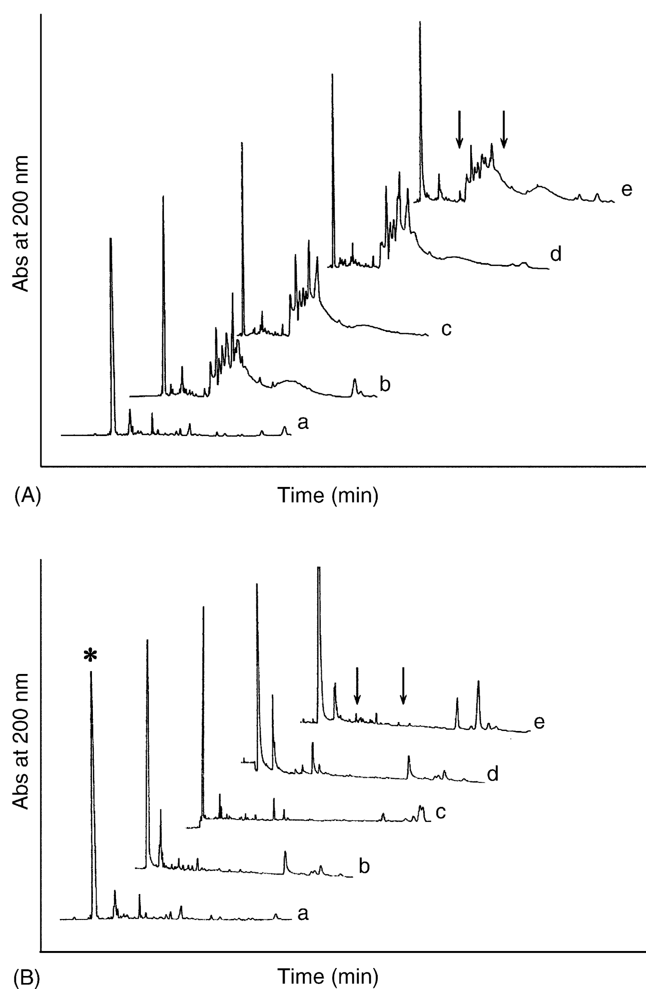

Fig. 1. (Panel A) Electropherograms obtained from urine of PD patients P_2, P_4, P_6 and P_8 (profiles b → e, respectively) and from a control (profile a). The arrows indicate the region of the profile that includes the cluster of peaks representing X-Pro dipeptides excreted in urine by these patients. (Panel B) Electrophoretic profiles obtained from urine of subjects with leg ulcerations but not affected by PD (profiles a → e, respectively). The peak indicated by an asterisk is that of endogenous creatinine. Experimental conditions: All runs were performed using fused silica capillaries of 50 cm effective length and 50 μm I.D. 50 mM sodium tetraborate pH 9.0 was used as background electrolyte and analytes were monitored at 214 nm. Samples (ca. 10 nL) were injected in pressure-injection mode operating at 25 °C and applying a voltage of 20 kV. For other details see the text.

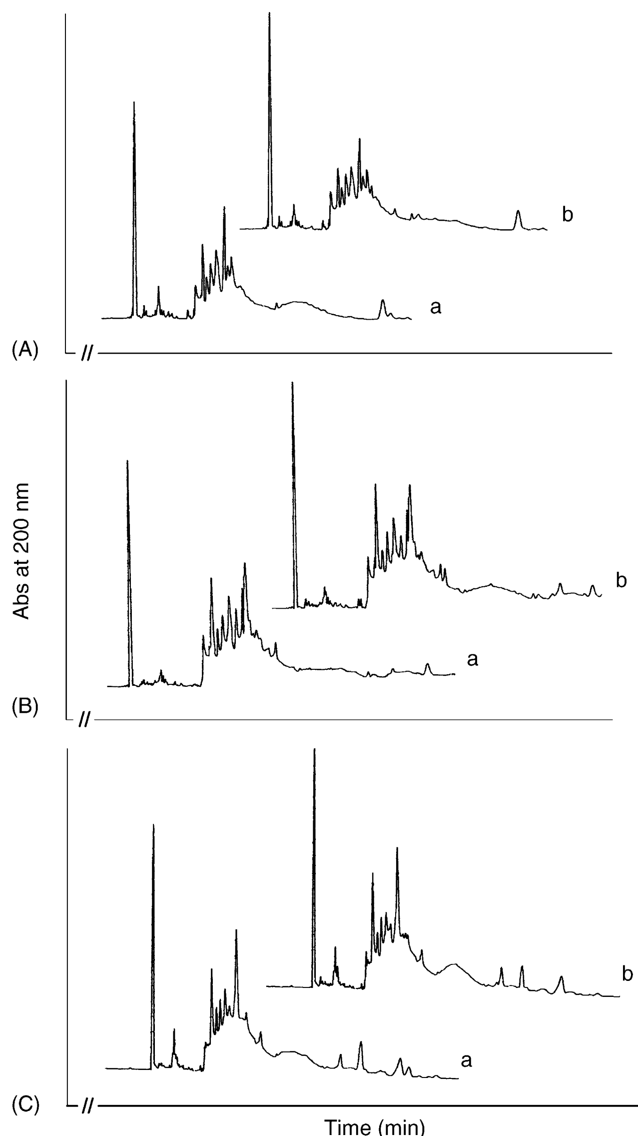

Fig. 2. Electrophoretic profiles obtained from urine of PD patient P_1 collected under fasting and non-fasting conditions (profiles a and b, respectively, in panel A); of PD patient P_3 collected early in the morning and late in the afternoon (profiles a and b, respectively, in panel B); of PD patient P_5 analyzed immediately after collection and after having been stored at −25 °C for a month (profiles a and b, respectively, in panel C). Experimental conditions as those described in the caption of Fig. 1.

question arose as to whether this cluster of peaks could vary as a function of a series of physico-chemical parameters. The availability of such a large number of specimens allowed to perform a series of experiments to answer this question. Detailed investigations have thus been performed on urine of patients collected (i) under different physiological conditions of patients or (ii) using different collection and storage procedures or (iii) after the samples had been submitted to different preanalytical manipulation steps. The electrophoretic profiles of a few patients, chosen at random among all investigated, have been reported in Fig. 2. As shown, only minor differences could be observed (i) in urine of patient P_1 collected either under fasting or non-fasting conditions (profiles a and b, respectively in panel A) or (ii) in urine of patient P_3 collected either early in the morning or late in the afternoon (profiles a and b, respectively in panel B) and (iii) in urine of patient P_5 analyzed immediately after collection and after having been stored frozen at −25 °C for a month (profiles a and b, respectively in panel C). Likewise, electrophoretic profiles obtained from urine of patient P_7 diluted, concentrated (15×) and reconstituted (after having liophylized an aliquot), were also found to be very similar to each other (Fig. 3, profiles a–c, respectively).

Moreover, since the qualitative difference between electrophoretic profiles of “non-PD” and “PD” patients, although tremendous, is not necessarily conclusive, nor does it exclude the possibility that other disorders may originate similar profiles, enzyme activity (against substrate Gly-Pro) using both capillary electrophoretic [25] and Chinard’s assay [19] was

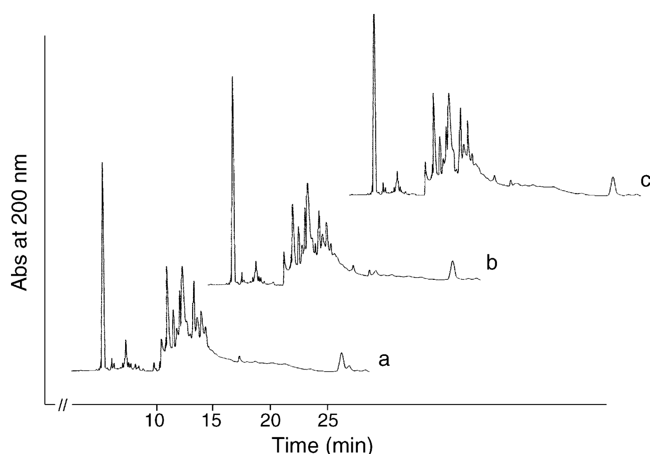

Fig. 3. Electrophoretic profiles obtained from urines of PD patient P_7 diluted, concentrated (15×) and reconstituted after having liophylized an aliquot (300 μL) (profiles a–c, respectively). To inject nearly-identical amounts of material in all cases, less than 1 nl of the concentrated sample was injected in the capillary. Experimental conditions as those described in the caption of Fig. 1.

detected on eryhtrocyte haemolysates of all 22 subjects to verify unambiguously the reliability of our method. The values detected in erythrocytes of subjects $P_1 \rightarrow P_{10}$ whose urines contained the complex mixture of imidodipeptides, ranged between 0.065 ± 0.009 mmol L^{-1} and 0.16 ± 0.01 mmol L^{-1} of Gly-Pro hydrolyzed min^{-1} g^{-1} heamoglobin, i.e. the levels known to be characteristics of PD patients [25]. Conversely, the values of prolidase activity calculated for the other 12 subjects (1.682 ± 0.33 mmol L^{-1} of Gly-Pro hydrolyzed min^{-1} g^{-1} heamoglobin) were practically identical to those of controls (1.701 ± 0.21 mmol L^{-1} of Gly-Pro hydrolyzed min^{-1} g^{-1} heamoglobin).

The unifying view that emerges from the data relative to determination of prolidase activity and identification of urinary dipeptides, indicates that CE is adequate to be considered an extremely powerful technique for diagnosis of prolidase deficiency because it is simple, efficient and reliable.

Recently, also ^{1}H NMR spectroscopy has been used to detect imidodipeptides in urine [42]. Using this technique the authors have analyzed three urine samples from PD patients. The assignement in patient's urine of the resonances of the proline and hydroxyproline protons as well as of the non-proline resonances of Val-Pro, Ala-Pro and Gly-Pro allowed the authors to indicate this as an efficient method to diagnose PD.

Given that the observed clinical phenotype in PD patients strongly correlates with the presence in urine of X-Pro/Hyp dipeptides, an efficient and reliable method for their determination obviously represents an essential tool for a correct diagnosis of the disorder. The applications that have been reported here show that the progressive establishment of new methodologies makes it easy to envision their clinical application in the near future since they meet the standards of reproducibility and performance that are expected of clinical tests.

Information concerning analytical procedures for diagnosing prolidase deficiency is summarized in Table 2.

Table 2
Techniques available for diagnosing prolidase deficiency

Technique	Source	Reference
Determination of prolidase activity		
Spectrophotometry at 515 nm	Erythrocytes, leukocytes, fibroblasts	[19,20]
RP-HPLC	Serum	[23]
CZE	Fibroblasts, erythrocytes	[24,25,27,28]
MALDI-TOF	Serum	[29]
Determination of imidodipeptides		
Amino acid analysis	Urine	[31–34]
Isotachophoresis	Urine	[35]
HPLC-API/MS	Urine, serum	[36–38]
CZE	Urine	[40]
MEKC	Urine	[41]
^{1}H NMR	Urine	[42]

3. Therapeutical approaches for prolidase deficiency

The main problem posed by all rare diseases is that they attract little interest from the pharmaceutical industry because they are infrequent; for this reason there is no treatment available for the majority of them [11]. This is the case of prolidase deficiency for which the only "treatment" currently applied is a topical conservative management of the severe skin ulcerations diffused on patients' lower limbs. Unfortunately, these attempts provide only very partial beneficial effects and are not resolutive. The following paragraphs provide a picture of all attempts so far performed on these patients in an effort to improve, at least, the quality of their daily lives.

3.1. Topical treatment

Since the main dermatological problem for prolidase deficient patients is represented by leg ulcers that are recalcitrant in healing, the topical treatment with appropriate ointments has been considered a plausible therapy for these lesions. In this context, ointments containing glycine and proline have been used by Arata et al. [14] and by Jenice et al. [15] on leg ulcers of two young (22 and 17 years old, respectively) patients. In particular Arata reported that a 40-day treatment with a combination of 5% glycine and 5% proline was effective in obtaining the epithelialization of a few ulcers that had previously resisted every therapeutical trial with antibiotics and other conventional ointments. An improvement of two lesions treated for 12 weeks with the same ointment mentioned above was observed also by Jenice, although he noticed that this treatment did not prevent the patient from developing additional ulcers elsewhere during the course of the study. Yasuda et al. [16] have studied the effects of corticosteroids on the skin lesions of two adult female siblings with prolidase deficiency. Their findings seem to indicate that imidodipeptides may play an important role in aggravating the skin lesions by priming neutrophil superoxide generation. The authors suggest that high-dose corticosteroids improve the ulcerations probably by inhibiting the infiltration and superoxide

generation by neutrophils. Monafo et al. [17] have reported that growth hormone (GH) deficiency was associated with prolidase deficiency in a 13 years old patient. The topical application of GH-containing ointment resulted in a complete, although transitory, healing of the ulcers. The positive effect of GH treatment was probably related to the physiologic role of this hormone in promoting the metabolism and growth of dermal connective tissue.

It should be mentioned here that also the oral administration of L-proline performed in numerous cases of PD [13,43–45] failed to relieve the dermatological lesions of these patients.

3.2. Enzyme replacement therapy

Many research groups have considered enzyme replacement as a mode of therapy for prolidase deficiency. In this respect, Ikeda et al. [46] performed adenovirus-mediated gene transfer of human prolidase cDNA into fibroblasts from patients with PD. Infection with the adenovirus vector carrying human prolidase cDNA resulted in an increase of prolidase activity in fibroblasts, up to approximately 7.5 times that of normal controls. Endo et al. [47] have performed on a PD patient an erythrocyte transfusion with 800 mL of whole blood. Immediately after this procedure the prolidase activity of the peripheral erythrocytes was elevated to approximately 35% of the normal values, although it gradually decreased, with an half-life of 41 days, to the low levels that are typical of these patients (between 5 and 9% of normal values). In an interesting study, Hechtman et al. [48] suggested that the prospect for successful treatment of PD using transfusion of affected persons with manganese-activated erythrocytes depends upon the capacity of the donor erythrocytes to hydrolyze imidodipeptides in relation to the amounts of substrates to which these cells would be exposed. Their results showed that inactive, or apo-prolidase, in human erythrocytes is responsive to exogenous Mn. They also showed that cells in which the active or holoenzyme form of prolidase has been formed retain high levels of enzymatic activity for at least 2 weeks. The results of their investigation allowed to conclude that the Mn-activated erythrocyte system could be considered as a serious candidate for clinical trial in cases of PD. Another case of blood transfusion on a PD patient has been described by Berardesca et al. [49]. This patient was submitted to a series of repeated transfusions (a total of six) and, if enzyme activity was initially raised to 15.7% of controls, its level declined to 12.7% after 8 days and around 4% 45 days following transfusions. Likewise, two other patients have been submitted "in parallel" by Lupi et al. [26] to apheresis exchanges repeated monthly for 4 consecutive month. Using CE, prolidase activity and urinary dipeptides were determined at regular intervals. An apparent, although transitory, improvement of ulcerations was observed and both patients tolerated the procedure well.

However, given the invasivity and the cost of transfusions, the possibility of using prolidase encapsulated in biodegradable particles or vescicles, has been recently evaluated, as a new approach for enzyme replacement therapy, in our laboratory.

The results of our experiments evidenced the positive role of microencapsulation as a process that provided stabilization of prolidase inside poly(D,L-lactide-co-glycolide) microspheres achieving release of the active enzyme both in vitro and ex vivo [50,51]. Since the therapeutic approach to prolidase deficiency through microsphere administration has never been performed before, our work demonstrated, at least, the feasibility of this preparation to carry small amounts of active prolidase inside cultured fibroblasts of PD patients ensuring a response to the intracellular accumulation of X-Pro dipeptides. A positive, although partial, result of the presence of active enzyme inside PD cells was an apparent improvement in fibroblast shape [51]. However, given the poor efficiency of this system for delivering prolidase into fibroblasts, further experiments have been performed to evaluate the possibility of utilizing liposomes as enzyme carriers. Thus, prolidase-loaded vescicles (prepared with a 1:1 egg L,*a*-phosphatidylcholine type XI-E: cholesterol with distearoylphosphatidylcholine-polyethylen glycol 2000) were prepared by means of a thin-film hydration method, that allows the preservation of enzymatic activity, and incubated with fibroblasts from PD patients [27]. When prolidase activity was assayed in the culture medium of the above fibroblasts and in washings of correspondent cell-layers, it was hardly detectable thus indicating that very low amounts, if any, of enzyme were released from liposomes in the medium. The versatility of CE allowed to prove easily the efficacy of liposomes in delivering prolidase intracellularly.

In fact, the electrophoretic profiles achieved from cellular extracts of PD patients' fibroblasts incubated, over different time spans with encapsulated prolidase, clearly evidenced the disappearance of the Gly-Pro peak with the progression of incubation time. As shown in Fig. 4, profiles a → d, the electropherogram obtained after 6-days incubation from cellular extracts of a PD patient, was practically identical to that of controls [27] thus confirming that exogenous

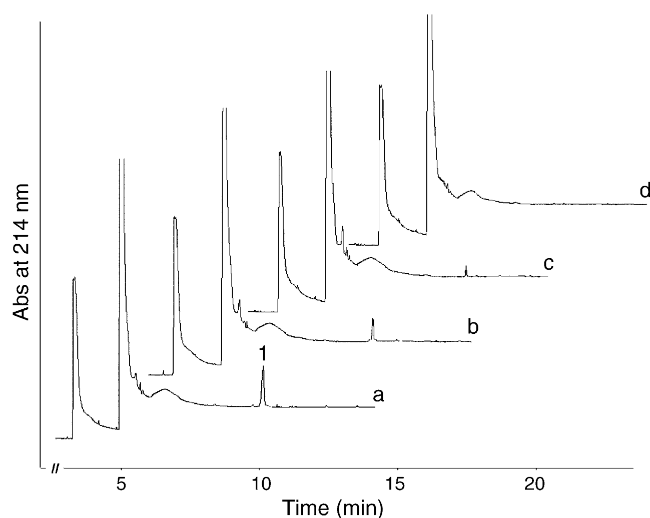

Fig. 4. Electrophoretic profiles showing the disappearance of endogenous dipeptide Gly-Pro (peak 1) upon incubation of fibroblasts from PD patient P_5 with liposome-encapsulated prolidase for 2, 4 and 6 days (profiles b–d, respectively). Profile (a) represents "time zero" of incubation reaction. Background electrolyte: 50 mM sodium tetraborate, pH 9.3 containing 30 mM α-cyclodextrins. Voltage applied: 30 kV. Temperature: 18 °C. All other experimental conditions as those described in the caption of Fig. 1.

Table 3
Therapeutical approaches for prolidase deficiency

Therapeutic approach	Drugs	Reference number
Topical treatment	Gly and Pro	[14,15]
	Corticosteroids	[16]
	Growth hormone	[17]
	L-Proline	[13,43–45]
Enzyme replacement	cDNA of human prolidase	[46]
	Fresh blood	[47]
	Fresh blood containing Mn activated erythrocytes	[48]
	Fresh blood	[49]
	Fresh blood	[26]
	Prolidase-loaded microspheres	[50,51]
	Prolidase-loaded liposomes	[27]

prolidase had been successfully transferred from liposomes to fibroblasts.

In conclusion, this study led to positive results both in the technologic and in the biologic areas. In fact, from the technologic stand point we were able to develop a reliable CE assay for the determination of prolidase activity in a very complex mixture. From the biologic stand point we designed a drug delivery system able to transfer active enzyme inside fibroblasts and to restore normal prolidase activity inside cells of patients.

Envisaged future clinical application of this system can be foreseen in an enzyme replacement therapy that can be achieved either by systemic administration of the liposomes formulation by parenteral route, or by direct application to patient skin ulcerations.

Information concerning all therapeutic approaches so far attempted is summarized in Table 3.

4. General considerations

In an effort to provide the whole picture of the techniques so far described for diagnosing prolidase deficiency and for developing a therapeutical approach, this article reports a survey of the literature concerning the utilization of different procedures in both fields.

The numerous publications cited in this article show the enormous progression achieved in the past 15 years with regard to the strategies developed for determining a correct diagnosis of this disorder. In particular, also in the light of our own experience, it seems plausible to suggest that CE, far from being complementary to other techniques, is certainly mature and adequate to be considered the method of choice for a routine use in dermatological divisions. The greatest merit of CE over other, even useful and reliable, approaches is that it provides simple and efficient separations, in a short time, with minimum consumption of reagent and at a relative high cost. Interestingly, whatever the chosen mode, CE is particularly attractive for the detection of dipeptides in urine because this matrix can be analyzed without being submitted to any preanalytical treatment. In addition, we have evidenced that CE screening of X-Pro dipeptides can be easily performed on diluted, freshly collected urine but that it can also be transported to the laboratory of reference frozen, thawed or even as a lyophilized powder, always producing the same results. It is apparent that such an easy technique provides an early diagnosis that, on its turn, may reduce the cascade of costs that are sure to arise later. In fact, since very often these patients die of general infections, the ability to rapidly diagnose PD could allow for the early implementation of appropriate infection control measures thus increasing their quality of life.

However, if the CE method may provide added security of diagnosis, a therapy that will benefit these patients still remains a dream. In this respect the efforts of several research groups and our own attempts to recreate intracellularly the deficient enzyme through an enzyme-replacement therapy may provide a solution, although not resolutive, to this problem. As far as our efforts are concerned, the role played by CE should be emphasized. Once again in fact this technique showed to be ideally suited for achieving information on the dynamics of this process. The data collected corroborate the hypothesis that the carrier system used is a successful candidate for replacing the missing enzyme. The fact that "normal" levels of prolidase activity have been almost completely restored in fibroblasts of all patients examined also indicates that, at least "in vitro", there are no potential adverse reactions to the vescicular system investigated. It should be underlined that, in case specific investigations would confirm a direct correlation between the build up of harmful imidodipeptides and the onset of skin ulcerations in PD patients, the enzyme-replacement strategy proposed represents the only concrete answer to this problem since it addresses the basic cause of the disease. Although it cannot be supposed that this therapeutic approach alone will meet all the expectations of PD patients, it seems plausible that a partial effect can be achieved, at least with respect to severe infections above mentioned.

5. Conclusions

Some may question the need for comprehensive efforts put in a direction that will benefit few patients. In our opinion this view is short-sighted since by researching this disease the opportunity increases to unlock the mystery of cell metabolism. For instance, in a series of interesting papers published by Palka et al., the levels of prolidase activity have been shown to correlate with collagen synthesis in Osteogenesis imperfecta [52,53], in pancreatic diseases [54] or in lung carcinoma planoepitheliale [55] and breast cancer MCF-7 cells [56–58]. These and other papers clearly indicate how important is the role of prolidase in collagen metabolism. It seems plausible that our efforts to define better the pathogenesis of prolidase deficiency may serve to gain further insight also into other (possibly correlated) severe disorders and vice versa. In light of this, the application of modern, available techniques is expected to make easier the improvement of knowledge regarding rare diseases and the full potential of these techniques still needs to be exploited.

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