Developmental forms of human skeletal-muscle AMP deaminase

The kinetic and regulatory properties of the enzyme*

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AMP deaminase isoforms from human skeletal muscle can be separated chromatographically [Kaletha, Spychała & Nowak (1987) *Experientia* 43, 440–443]. In adult tissue nearly all the AMP deaminase activity was eluted from phosphocellulose with 0.75 M-KCl ('adult' isoform), and the remaining activity could be eluted with 2.0 M-KCl. Conversely, most of the AMP deaminase activity from 11-week-old fetal tissue was eluted from phosphocellulose with 2.0 M-KCl ('fetal' isoform). In the present paper the kinetic and regulatory properties of AMP deaminase extracted from 11- and 16-week-old fetal skeletal muscle are reported. The two isoforms from 11-week-old human fetus differed distinctly in these properties. The 'fetal' isoform had about 5-fold higher half-saturation constant ($S_{0.5}$) value than the 'adult' form. It was also more sensitive to the influence of some important regulatory ligands (ADP, ATP and P_i), and exhibited a different pH/activity profile. The 'adult' isoform of AMP deaminase from fetal muscle and the enzyme from mature muscle possessed similar kinetic and regulatory properties. This isoform seems not to be subject to any major modifications during further ontogenesis. This is not true, however, for the 'fetal' isoform. In the muscle of 16-week-old human fetus, the 'fetal' isoform showed a peculiar, biphasic, type of substrate-saturation kinetics. This phenomenon may reflect appearance of the next, developmentally programmed, isoform of human skeletal-muscle AMP deaminase.

INTRODUCTION

AMP deaminase (EC 3.5.4.6), the enzyme catalysing the irreversible hydrolytic deamination of AMP to IMP and ammonia, is widely distributed among mammalian cell types. In skeletal muscle, the activity of the enzyme is much higher than in other tissues [1], and it is well established that the ammonia produced by working muscle arises from AMP deamination [2].

Although the physiological function of AMP deaminase is not yet fully understood, it appears to play a role in regulation of relative concentrations of intracellular purine nucleotide pools [3], the stabilization of adenylate energy charge [4], the replenishment of citric acid cycle intermediates [5] and the deamination of amino acids via the purine nucleotide cycle [6].

AMP deaminase from different sources varies in qualitative and quantitative aspects of interaction with modifiers, with each form of the enzyme exhibiting properties representative of an allosteric system. Thus far, the enzyme has been studied most thoroughly in rabbit [7–10] and rat [11–14] skeletal muscle.

At least three isoforms of AMP deaminase have been identified in rat tissues on the basis of immunological, physical and kinetic properties [15,16]. Isoform A is expressed in skeletal muscle and diaphragm, isoform B in kidney, and isoform C in heart muscle. On the basis of these same criteria, four AMP deaminase isoforms, E1, E2, L and M, found in erythrocytes, liver and skeletal muscle respectively, have been reported to exist in various human tissues [17]. Isoform E1 has been found to be immunologically similar to isoform E2. Isoforms E1, M and L were immunologically different from one another, but they were similar in this respect to rat isoforms C, A and B respectively.

The total amount of AMP deaminase in skeletal muscle varies distinctly during the course of animal development [18,19], and the observed changes in the properties of the enzyme [19,20] suggest that a developmental shift in the muscle isoenzyme pattern may occur.

Here we describe the kinetic and regulatory properties of two developmental isoforms of AMP deaminase which have been recently reported [21] to exist in skeletal muscle of the human fetus.

MATERIALS AND METHODS

Isolation of the enzyme

Samples of human fetus leg muscle at 11 and 16 weeks of gestation were obtained from the Obstetrics and Gynaecological Department of the Medical School in Gdańsk. Adult human thigh-muscle samples were obtained from the City Hospital in Starogard Gdański. All fetal specimens were processed within 2–4 h, whereas the adult human muscle samples, taken in the course of surgery, were frozen and processed after several days. After removal of the skin and connective tissue, samples (weighing 3–8 g) were washed, homogenized in 15–20 vol. (v/w) of extraction buffer [9], and AMP deaminase was isolated by chromatography on phosphocellulose, by the procedure of Smiley *et al.* [9]. The enzyme adsorbed on phosphocellulose was eluted stepwise, by washing the column with 0.75 M-KCl and then 2.0 M-KCl. The AMP deaminase in extracts obtained from both fetus and adult human skeletal muscle was eluted from the column in two well-separated peaks of activity [21], indicating the presence of at least two different molecular forms of the enzyme. In 11-week-old fetal

* Dedicated to the memory of Professor Włodzimierz Mozłowski (1895–1975).

Table 1. Effect of ATP, ADP and P_i on the kinetic parameters of the reaction catalysed by molecular forms of AMP deaminase from 11-week-old human fetal skeletal muscle

The data are means of five experiments, with S.E.M. values in parentheses.

Effector added	'Adult' form			'Fetal' form		
	$s_{0.5}$ (mM)	$V_{max.}$ (% of control)	h	$s_{0.5}$ (mM)	$V_{max.}$ (% of control)	h
None (control)	0.55 (0.05)	100	1.1	2.38 (0.45)	100	1.1
1 mM-ATP	0.33 (0.03)	82	1.2	0.52 (0.05)	83	1.0
1 mM-ADP	0.30 (0.06)	65	1.2	0.80 (0.10)	47	1.3
2.5 mM- P_i	0.73 (0.05)	69	1.3	1.08 (0.10)	25	1.4

muscle, most of the AMP deaminase activity was eluted with 2.0 M-KCl ('fetal' isoform) whereas in 16-week-old fetal tissue most of the enzyme activity was eluted with 0.75 M-KCl ('adult' isoform). In adult human skeletal muscle, almost all AMP deaminase activity was eluted with 0.75 M-KCl; the 'fetal' isoform constituted only a very small part of total enzyme activity in that tissue.

The specific activity of the preparation obtained (measured at 10 mM-AMP and 30 °C) was 1.3 ± 0.3 and 17.4 ± 1.2 $\mu\text{mol}/\text{min}$ per mg of protein for 'adult' and 'fetal' isoforms of 11-week-old fetal muscle enzyme respectively ($n = 5$), 6.2 ± 0.5 and 7.1 ± 0.7 $\mu\text{mol}/\text{min}$ per mg for 'adult' and 'fetal' isoforms of 16-week-old fetal muscle enzyme respectively ($n = 3$), and 31.2 ± 4.2 $\mu\text{mol}/\text{min}$ per mg for 'adult' isoform obtained from adult human skeletal-muscle extract ($n = 3$). The appropriate relative activities of the two AMP deaminase isoforms (the ratio of the specific activity of the 'fetal' isoform to that of the 'adult' one) were about 13 and 1 for the enzyme from 11- and 16-week-old skeletal muscle respectively.

Enzyme assay

AMP deaminase activity was measured colorimetrically by the phenol/hypochlorite method of Chaney & Marbach [22]. The incubation medium, in a final volume of 0.5 ml, contained 0.05 M-potassium succinate buffer, pH 6.5, 100 mM-KCl and various concentrations of the substrate and effectors. After equilibration of the medium at 30 °C, 25 μl of enzyme solution was added to start the reaction. The initial velocity of the reaction was determined from the mean amount of ammonia liberated in three parallel incubations. No symptoms of enzyme denaturation was observed, as judged from the proportionality of the ammonia liberation versus time for the period of the reaction course (15 min).

When 'adult' isoforms of 16-week-old fetus and adult human enzyme were examined at low (75 μM) substrate concentration, the spectrophotometric method [23] was used to monitor enzyme activity. The decrease in A_{265} for 2 min was monitored with a Specord UV-vis spectrophotometer fitted with a constant-temperature cell housing. From a 2 min tracing of the A_{265} change, the initial velocity of the reaction was calculated by using the molar coefficient given by Smiley & Suelter [8]. The reaction mixture, in a final volume of 3 ml, contained 0.05 M-potassium succinate buffer, pH 6.5, 100 mM-KCl, 75 μM -AMP and different concentrations of various

effectors. After equilibration at 30 °C, 25 μl of enzyme solution was added to the incubation medium to start the reaction. The measurements were carried out against a blank containing all the additions except substrate.

Protein was determined with Coomassie Brilliant Blue [24].

Calculation of kinetic parameters

The kinetic parameters of the reaction (maximum velocity of the reaction, $V_{max.}$, half-saturation constant, $s_{0.5}$, and co-operativity coefficient, h) were calculated on a microcomputer, by using a BASIC program based on the flow diagram described by Atkins [25]. The fundamental part of this program is a subroutine which performs a series of iterative regression steps for a given preliminary value of $V_{max.}$. In the course of these iterations, the logarithmic form of the Hill equation was fitted directly to the experimental kinetic data by minimizing the sum of the squares of residuals.

Reagents

5'-AMP (type V, from horse skeletal muscle), ADP, ATP (Na^+ salts) and GTP (Li^+ salt) were supplied by Sigma (St. Louis, MO, U.S.A.); CoA (Li^+ salt) and activated fatty acids were purchased from Calbiochem-Boehringer (La Jolla, CA, U.S.A.); cellulose phosphate was from Whatman (Maidstone, Kent, U.K.). All other chemicals were from Polskie Odczynniki Chemiczne (Gliwice, Poland).

RESULTS

The two molecular forms of AMP deaminase obtained from 11-week-old human fetal muscle possessed distinctly different kinetic and regulatory properties. In the presence of 100 mM-KCl and no nucleotide effectors (control conditions), the reaction catalysed by 'adult' isoform showed hyperbolic-type kinetics ($h = 1.1$), reaching saturation velocity at about 3 mM-AMP. Under the same conditions, the 'fetal' isoform needed nearly 5 times higher substrate concentration to reach its saturation velocity. This same difference had been calculated for half-saturation constants (Table 1).

For both isoforms, the addition of 1 mM-ATP stimulated the reaction at lower substrate concentrations, and inhibited at higher ones. The stimulatory effect was much more pronounced, however, for the 'fetal' isoform. In 11-week-old fetal muscle, 1 mM-ATP decreased the $s_{0.5}$

Fig. 1. Effect of substrate concentration on velocity of the reaction catalysed by 'adult' (a) and 'fetal' (b) isoforms of 16-week-old human fetal skeletal-muscle AMP deaminase

In the presence of 100 mM-KCl only (●) and with the addition of 1 mM-ADP (▼), 1 mM-ATP (▲) or 2.5 mM- P_i (■).

value of the 'adult' isoform nearly 2-fold, but caused a 4.5-fold decrease in the 'fetal' one (Table 1). ADP (1 mM) decreased the $s_{0.5}$ value for the 'adult' isoform 2-fold and that for the 'fetal' isoform 3-fold. In addition to enhancing the enzyme-substrate affinity, ADP at higher concentrations distinctly decreased the maximum velocity of the reaction of both molecular forms of AMP deaminase. P_i inhibited the activity of 11-week-old human fetal muscle AMP deaminase isoforms at each substrate concentration tested, mainly by influencing the maximum velocity of the reaction (Table 1).

Fig. 1(a) shows the substrate-saturation curves for the reaction catalysed by 'adult' isoform of 16-week-old human fetal muscle AMP deaminase. In the presence of 100 mM-KCl this isoform followed regular hyperbolic kinetics ($h = 1.1$), as it did in 11-week-old muscle. Unlike the enzyme isolated from 11-week-old fetal muscle, however, the 'adult' isoform from 16-week-old muscle was only minimally affected by 1 mM-ATP (Table 2); 1 mM-ADP halved its half-saturation constant (from 0.5 to 0.25 mM), whereas 2.5 mM- P_i more than doubled it (to 1.09 mM). Neither, however, had any effect on the maximum velocity of the reaction (Table 2).

Fig. 1(b) illustrates the kinetic and regulatory properties of the 'fetal' isoform of AMP deaminase from 16-week-old human fetal muscle. Two of the curves (control and that obtained in the presence of 2.5 mM- P_i) possess a distinct inflection point, and may be considered to be composed of two overlapping curves, suggesting the presence of two enzyme subforms in the 'fetal' isoform preparation of 16-week-old human fetus muscle. Reciprocal plots ($1/v$ versus $1/[S]$ and $v/[S]$ versus v ; results not shown) are consistent with this hypothesis and demonstrate clearly the two-component character of these curves. Hence, each of the velocity curves in Fig. 1(b) represents the activity, which is the sum of the activities of two co-existing kinetically different subforms of AMP deaminase. In contrast with the sub-form operating with full activity in the lower range of substrate concentrations, the second one seems to be a regulatory protein, and under control conditions it exhibits a sigmoidal substrate-saturation profile. Addition of 2.5 mM- P_i to the incubation medium strongly inhibited the activity of 'fetal' isoform of AMP deaminase from skeletal muscle of 16-week-old human fetus at each

Table 2. Effect of ATP, ADP and P_i on the kinetic parameters of the reaction catalysed by molecular forms of AMP deaminase from 16-week-old human fetal skeletal muscle

The data are means of three experiments, with S.E.M. values in parentheses. The values with asterisks represent the kinetic parameters, calculated from the equation:

$$v = V_1/(1 + K_m/[S]) + V_2/\{1 + (K_{0.5}/[S])^n\}$$

by using a non-linear regression method, for the two components of the biphasic kinetic curves.

Effector added	'Adult' form			'Fetal' form		
	$s_{0.5}$ (mM)	V_{max} (% of control)	h	$s_{0.5}$ (mM)	V_{max} (% of control)	h
None (control)	0.50 (0.06)	100	1.1	0.3*, 4.1**	56*, 100**	1.0*, 2.3**
1 mM-ATP	0.46 (0.04)	98	1.3	1.0	134	1.1
1 mM-ADP	0.25 (0.06)	97	1.0	1.5	93	0.9
2.5 mM- P_i	1.09 (0.08)	97	1.4	0.6*, 10.6**	31*, 61**	1.0*, 7.8**

Table 3. Effect of ATP, ADP and P_i on the kinetic parameters of the reaction catalysed by AMP deaminase from adult human skeletal muscle

The data are means of three experiments, with S.E.M. values in parentheses.

Effector added	'Adult' form		<i>h</i>
	$s_{0.5}$ (mM)	V_{max} (% of control)	
None (control)	0.65 (0.06)	100	1.1
1 mM-ATP	0.78 (0.04)	96	1.2
1 mM-ADP	0.65 (0.03)	96	1.0
2.5 mM- P_i	1.03 (0.07)	98	1.2

substrate concentration tested. In the presence of this inhibitor the two-component character of the kinetic curve is even more visible, and the inflection point, which under control conditions occurred at about 2 mM-AMP, is now located at about 9 mM concentration of substrate (Fig. 1b). ATP (1 mM) strongly activated the 'fetal' isoform of 16-week-old fetal muscle AMP deaminase over the whole range of substrate concentrations examined. Interestingly, the distribution of experimental data points in the presence of this nucleotide was nearly regular, and the inflection point was hardly detectable. The same seems to be true when 1 mM-ADP was added to the reaction mixture. In contrast with ATP, however, ADP did not change the maximum velocity of the reaction (Fig. 1b).

Table 2 presents the kinetic parameters calculated for the reaction catalysed by the 'fetal' isoform of AMP deaminase from the skeletal muscle of 16-week-old human fetus in the absence and in the presence of the effectors tested. Under control conditions, as well as for the reaction examined in the presence of 2.5 mM- P_i , the appropriate values of $s_{0.5}$ and h and the relative values of the maximum velocity of the reaction were calculated for both components of the biphasic velocity curves.

In the presence of 100 mM-KCl only, the reaction catalysed by adult human skeletal-muscle AMP deaminase revealed a regular hyperbolic type of kinetics, with the $s_{0.5}$ value as low as 0.65 mM (Table 3). The addition of 1 mM-ADP to the incubation medium had no effect on the enzyme activity, whereas addition of 1 mM-ATP or 2.5 mM- P_i slightly inhibited the activity of the enzyme, affecting $s_{0.5}$, but not V_{max} , or h .

The effect of some important regulatory ligands on AMP deaminase activity from 16-week-old fetal muscle is shown in Figs. 2(a) and 3(a). Both molecular forms of fetal muscle AMP deaminase exhibited qualitatively and quantitatively different properties in the presence of these ligands. ATP strongly stimulated the 'fetal' isoform of 16-week-old fetal muscle enzyme, but had the opposite effect on the activity of the 'adult' isoform. In contrast, ADP activated and GTP and P_i inhibited the activity of both isoforms extracted from 16-week-old human fetal muscle. Interestingly, the inhibition of the 'fetal' isoform by GTP decreased with effector concentrations higher than 0.1 mM. At adenylate concentrations as low as 75 μ M (Fig. 3a), however, no activation by ADP (at least

Fig. 2. Influence of ADP (▼), ATP (▲), GTP (■) and P_i (●) on the activity of (a) 'adult' (—) and 'fetal' (---) isoforms of 16-week-old human fetal skeletal-muscle AMP deaminase and (b) adult human skeletal-muscle AMP deaminase

The reaction was carried out at 0.6 mM-AMP concentration when the 'fetal' isoform was investigated; otherwise AMP concentration was 0.3 mM. v_0 is the enzyme activity measured in the presence of 100 mM-KCl only. For nucleotide effectors the upper line of values, and for P_i the lower line, on the abscissa are valid.

for effector concentrations up to 25 μ M) occurred. At the same low concentration of the substrate, P_i and the two remaining nucleotide effectors had a pronounced inhibitory effect.

AMP deaminase extracted from adult human skeletal muscle exhibited very similar properties to the fetal muscle 'adult' isoform. The most distinguishing difference concerned the effect of ADP, which stimulated

Fig. 3. Influence of ADP (▼), ATP (▲), GTP (■), P_i (●) and palmitoyl-CoA (△) on the activity of (a) 'adult' isoform of 16-week-old human fetal skeletal-muscle AMP deaminase and (b) adult human skeletal-muscle AMP deaminase

The reaction was carried out at $75 \mu\text{M}$ -AMP. v_0 is the enzyme activity measured in the presence of 100 mM -KCl only. For nucleotide effectors and palmitoyl-CoA the upper line of values, and for P_i the lower line, on the abscissa are valid.

adult human muscle enzyme (Fig. 2b) at concentrations as low as $50 \mu\text{M}$, i.e. much lower than has been found for the 'adult' isoform of fetal muscle AMP deaminase (Fig. 2a). Nevertheless, in the presence of very low concentrations of adenylate, the activation by ADP was hardly detectable for effector concentrations up to $25 \mu\text{M}$ (Fig. 3b).

Table 4 presents the effect of CoA and CoA derivatives

of short- and long-chain fatty acids on the activity of molecular forms of skeletal-muscle AMP deaminase. The modulatory effect exerted by the activated fatty acids tested was found to be dependent not only on the chemical nature and concentration of the effector, but also on the substrate concentration. Activated long-chain fatty acids inhibited the 'adult' isoform of AMP deaminase extracted from 11- and 16-week-old human fetal muscle and from adult human skeletal muscle. At $75 \mu\text{M}$ substrate (see Fig. 3), the strong inhibition, even at very low concentrations of activated fatty acids, was especially distinct for adult human skeletal-muscle enzyme.

The two isoforms of 11- and 16-week-old human fetal skeletal-muscle AMP-deaminase have been found (results not shown) to be different with respect to their pH-dependent activity modulation. The enzyme extracted from less mature fetal tissue (especially its 'fetal' isoform) showed a marked decrease in the activity at the acidic side of the pH optimum (pH 6.6 for 'adult' isoform and 6.8 for 'fetal' isoform). The decrease in enzyme activity at the alkaline side of the optimum was much less pronounced. In contrast with that, the AMP deaminase extracted from 16-week-old human fetal skeletal muscle showed a more marked decrease in the activity at the alkaline side of its optimum (pH 6.4 for both isoforms). Again, the 'fetal' isoform of the enzyme was found to be more sensitive to pH-induced activity modulation. AMP deaminase from adult human tissue had a pH/activity profile similar to that of the 'adult' isoform of 16-week-old fetal skeletal muscle.

DISCUSSION

We previously reported [19] that AMP deaminase from 14-day-old chick-embryo skeletal muscle changed its catalytic properties differently from the enzyme from adult animal tissue in response to temperature. We hypothesized that different catalytic properties of AMP deaminase in the tissues studied reflected a different isoenzyme composition in developing chick muscle. In 1978, Sammons & Chilson [20] presented data demonstrating the occurrence of an isoenzyme change during the development of chick skeletal muscle. Subsequent studies performed in our laboratory [26–28] showed that the enzyme obtained from 14-day-old chick embryo differed in many kinetic and regulatory properties from that obtained from adult hen muscle.

The existence of a developmental shift of AMP deaminase isoenzyme composition in several rat tissues was first reported by Ogasawara *et al.* [29]. They concluded that skeletal muscle, in contrast with heart and liver, contained mostly the isoenzyme A form of AMP deaminase throughout postnatal animal development, but that the skeletal muscle of 3-day-old rat transiently contained another isoenzyme. Thus an isoenzyme composition change might also occur in rat muscle tissue. This hypothesis might be consistent with the different catalytic response to temperature which has been found in skeletal-muscle extracts of newborn and older rats [30]. Most recently, Marquetant *et al.* [31] have demonstrated that there are three developmental isoforms of rat skeletal-muscle AMP deaminase, which are presumably the result of developmentally controlled changes in gene expression.

AMP deaminase from human skeletal muscle was first

Table 4. Effect of CoA and some activated fatty acids on the activity of AMP deaminase from fetal and adult human skeletal muscle

The activity measured under control conditions is set at 100. The enzyme activity was measured at 0.3 mM- and 0.6 mM-AMP when 'adult' and 'fetal' isoforms were studied respectively.

Effector added (μM)			Relative velocity (%)	
			'Adult' isoform	'Fetal' isoform
11-week-old human fetus	Palmitoyl-CoA	(2.5)	93	101
		(5)	85	99
		(15)	77	97
		(30)	72	95
	Oleoyl-CoA	(30)	67	91
	Acetyl-CoA	(30)	85	109
	CoA	(30)	105	138
16-week-old human fetus	Palmitoyl-CoA	(2.5)	98	115
		(5)	83	110
		(15)	64	90
		(30)	45	58
	Oleoyl-CoA	(30)	34	29
	Acetyl-CoA	(30)	78	91
	CoA	(30)	101	99
Adult man	Palmitoyl-CoA	(2.5)	105	
		(5)	95	
		(15)	71	
		(30)	35	
	Oleoyl-CoA	(30)	26	
	Acetyl-CoA	(30)	113	
	CoA	(30)	119	

purified and characterized by Makarewicz & Stankiewicz [32]. Their studies, as well as those published later [17,33,34], were performed on samples of human muscle taken during routine autopsy, i.e. at least 24 h after death. The experiments in the present paper were performed on samples of adult human muscle taken during surgery and immediately frozen. This procedure, especially in view of data published by Ranieri-Raggi & Raggi [35,36], indicating modification of the enzyme activity by proteolysis, should allow us to obtain a more realistic description of the kinetic and regulatory properties of AMP deaminase.

It had been found by Makarewicz & Stankiewicz [32] that AMP deaminase extracted from adult human skeletal muscle resembled the enzyme from rabbit and rat muscle in many properties. It showed a rather sharp pH optimum at 6.5 and an absolute requirement for univalent cations. The highest activity was observed in the presence of 150 mM-KCl, at which the enzyme showed regular hyperbolic kinetics, with a half-saturation constant as high as 1 mM. P_i inhibited and ATP activated the enzyme. The last effect diminished markedly, however, when K^+ was present in the incubation medium. Our results with the enzyme extracted from adult human muscle are in general consistent with these findings.

Recently, we have reported the existence of two chromatographically separable forms of AMP deaminase in fetal skeletal muscle [21], which differed distinctly in substrate specificity and stability. In contrast with the 'adult' isoform, the 'fetal' one was very unstable, quickly losing its activity even if kept at 4 °C. This

isoform has also been detected in adult human skeletal muscle, but only if the tissue sample was taken during surgery. It was undetectable in the muscle sample taken during autopsy. The 'adult' isoform of fetal muscle AMP deaminase seems to be very similar to the enzyme typical of adult human muscle. In the presence of 100 mM-KCl, it exhibited regular hyperbolic kinetics, with the $s_{0.5}$ value close to 0.6 mM. This agrees very well with the estimate by Ogasawara *et al.* [17] for human isoenzyme M. Moreover, both the 'adult' isoform of fetal muscle AMP deaminase and the enzyme from adult human tissue seem to be similarly regulated by several important modulatory ligands. This suggests that the 'adult' isoform of fetal muscle enzyme (especially that from 16-week-old fetal muscle) is probably not subject to any major modifications in respect to kinetic and regulatory properties during further stages of human ontogenesis. The same seems not to be true for the 'fetal' isoform. This form, in contrast with the 'adult' one, gradually decreased in specific activity during prenatal human ontogenesis, and has been found to be very susceptible to ligand-induced activity modulations.

In the muscle of 16-week-old human fetus, the 'fetal' isoform of AMP deaminase manifested a peculiar biphasic type of kinetics. A detailed analysis of the kinetic data obtained allowed us to interpret this phenomenon as the effect of simultaneous action of at least two, kinetically distinguishable, molecular subforms of the enzyme, which were eluted from the column at approximately the same ionic strength. According to Segel [37], the presence of multiple forms of the enzyme is easy to detect if the $V_{\max}$ and K_m of one form are

significantly different from those of the other. If additionally a similar significant difference in their $V_{\max}/K_m$ ratios exists, the unequal contribution of the two enzyme forms to the observed initial velocity in the first-order region of the substrate concentration is clearly indicated by the obvious inflection in the (total) velocity curve plot, and by a distinct curvature of the reciprocal plots. We have observed a similar behaviour of the 'fetal' isoform preparation obtained from 16-week-old human fetal skeletal muscle. The calculated values of $V_{\max}/s_{0.5}$ ratios, which in control conditions were 12.2 and 1.4 (i.e. 8.8:1), for low- $s_{0.5}$ and high- $s_{0.5}$ components of the total-velocity curve respectively, in the presence of 2.5 mM- P_i changed to 5.9 and 0.16 (i.e. 35.9:1) for low- $s_{0.5}$ and high- $s_{0.5}$ components of the total velocity curve respectively. This change made the inflection in the total-velocity curve (Fig. 1b) even more distinct than that in the curve obtained for control conditions. ATP and ADP acted in an opposite way to P_i : in the presence of these effectors the inflection in the total-velocity curve (Fig. 1b) was hardly detectable.

It has been postulated by Marquetant *et al.* [31] that three developmental isoforms, produced sequentially during ontogenesis in rat skeletal muscle, indicate the existence of a developmentally controlled schedule of AMP deaminase gene expression. The same appears to be true for human muscle AMP deaminase.

In our previous paper we postulated [21] that patients with myoadenylate deaminase deficiency [38,39] probably have no 'adult' isoform in their muscle tissue, and that the remnant activities found in the muscle biopsies of the patients with this deficiency would consist of the 'fetal' isoform only. The cloning of skeletal-muscle AMP deaminase gene [40] may be very helpful in searching for the molecular basis of the myoadenylate deficiency syndrome.

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