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6-PYRUVOYL-TETRAHYDROPTERIN REDUCTASES

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Introduction

Sepiapterin reductase has been proven by specific suppression of the enzyme activity (1,2) to play an obligate role in the biosynthesis of tetrahydrobiopterin (BH_4). However, the physiological function of 6-pyruvoyl tetrahydropterin reductase (PPH_4R), which directly competes with sepiapterin reductase for the utilization of 6-pyruvoyl tetrahydropterin (PPH_4), still remains to be clarified (3,4). This is of particular interest, since 6-lactoyl tetrahydropterin, the product of PPH_4R, is readily reduced to BH_4 by sepiapterin reductase (1,2,4-7). From the viewpoint of its wide occurrence (8-11), ranging from the fruitfly (8) to human (11) tissues, PPH_4R appears to have been conserved during evolution, which might indicate an essential physiological role.

Taking into consideration the similar affinities of PPH_4R and sepiaterin reductase for PPH_4 and the comparable conversion rates of the latter (3,5), we first attempted to elucidate the contribution of PPH_4R to the BH_4 biosynthesis. Due to a lack of adequate inhibitors, we isolated the reductase from human liver and purified it to apparent homogeneity in order to produce monoclonal antibodies to PPH_4R.

Milstien and Kaufman, referring to their results obtained from inhibition studies and analysis of the amino acid composition, recently suggested that PPH_4R from rat brain is a unique type of keto reductase in the group of aldo-keto reductases (4). This conclusion, however, does not agree with our results.

Results

During purification (12) the enzyme was identified by its ability to convert PPH_4 to 6-lactoyl tetrahydropterin, which can be clearly distinguished from 6-(1'-hydroxy-2'-oxopropyl)-tetrahydropterin by use of reversed phase HPLC with electrochemical detection (11).

Following immunization, fusion, and subcloning, three monoclonal antibodies were obtained, which specifically recognize PPH_4R in ELISA, immuno dot blot, and Western blot. Cross-reactivity with human sepiapterin reductase was not observed. The employment of the monoclonal antibodies in further immunoreactivity tests, however, revealed an unexpected result as will be discussed below. Similar physical and biochemical properties of other oxidoreductases described in the literature led to the comparison of PPH_4R with human brain carbonyl reductase (13) and aldose reductase (14), as well as with aldehyde reductase from human liver (15). Using different polyclonal antisera to these oxidoreductases (16) in a dot immuno blot assay with positive controls and PPH_4R as antigen, structural similarities between the latter and aldose reductase were detected. The observed cross-reactivity was further confirmed by the results obtained from a radial double-

immunodiffusion with anti-aldose reductase serum and a Western blot analysis applying the monoclonal antibodies to PPH_4R.

Studies on the substrate specificity of PPH_4R also yielded catalytic properties in close agreement with those reported for aldose reductase, while the latter quantitatively reduces PPH_4 to 6-lactoyl tetrahydropterin (17).

So far we have not been able to deal with the blocked N-terminal amino acid of PPH_4R and to perform the sequence analysis.

Table 1: Comparison of Aldose Reductase and PPH_4 Reductase

	Aldose Reductase	PPH_4 Reductase
Origin	human brain	human liver
MW	38'000 Da	35'000 Da
Ip	5.9	5.9
Coenzyme	NADPH	NADPH
H-transfer	*pro*-R	*pro*-R
Substrates	aldoses	aldoses
	PPH_4	PPH_4
	others	others
Immunology	corresponding immunoreactivity	

Conclusion

Although the data of the amino acid sequence of PPH_4R are not available yet, the essential immunochemical and biochemical properties of the enzyme, partly listed in Tab. 1 and shortly to be published in detail (17) suggest that PPH_4R from human liver and aldose reductase from human brain are identical enzymes or at least isoenzymes.

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