BIOLOGY OF REPRODUCTION 36, 125–129 (1987)

Amino Acid Content of Preimplantation Rabbit Embryos and Fluids of the Reproductive Tract[1]

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ABSTRACT

As a companion to amino acid transport and protein synthetic studies, it was of interest to quantify the amino acid pools in embryos and reproductive tract fluids during preimplantation development. Primary amines in the acid-soluble extracts of embryo and fluid samples were separated by high-performance liquid chromatography, reacted with o-phthalaldehyde, and quantified by fluorescence emission. The amino acid compositions of embryos were like those of corresponding reproductive tract fluids. Taurine was high in eggs and fluids but declined with development, while glycine levels rose. Glycine was highest in concentration in all samples (except the egg), followed by glutamate and alanine, while most other amino acids were consistently of low abundance.

INTRODUCTION

The highly sensitive o-phthalaldehyde reaction method (Benson and Hare, 1975), previously used to quantify amino acid pools in mouse preimplantation samples (Schultz et al., 1981), has been employed in the present work to quantify the free amino acid content and composition of rabbit samples of unfertilized eggs, morulae, blastocysts, and corresponding reproductive tract fluids. Such information is important to establishing the context in which changes in amino acid transport and protein synthesis take place.

MATERIALS AND METHODS

Recovery of Embryo and Reproductive Tract Fluid Samples

Reproductive age (approximately six-mo-old) virgin Dutch Belted rabbits were superovulated. Eggs, morulae (69 h after administration of luteinizing hormone [LH]), and blastocysts were collected in amino acid-free Medium 2 (M2) as described previously (Miller and Schultz, 1983). Blastocysts, free of M2, were punctured in 0.5 ml Eppendorf tubes and subjected to centrifugation at 10,000 × *g* for two min. The supernatant was saved for amino acid analysis. Eggs, morulae and blastocyst cellular material were washed in 500 μl of M2, and the supernatant was drawn off after centrifugation. In pilot studies, quantification of serial washes revealed insignificant content of free amino acid, which suggested minimal loss of amino acid from the cellular material with this protocol. Triple distilled water (50 μl) was added to each tube, and all cellular material was subjected to three freeze-thaw-vortex cycles to disrupt and lyse the cells. Samples were subjected to lyophilization and redissolved in 5 μl of triple distilled water. For blastocyst cavity fluid, a 5-μl aliquot or a 5-μl aliquot of a one in ten dilution of the fluid was used for the subsequent analysis.

Samples of oviduct and uterine fluid contents from nonsuperovulated mated rabbits 1, 3, and 6 days post coitus were also prepared. For Day one and Day six oviducts, 1.0 ml of M2 medium was flushed gently through the oviduct from the fimbrial end, and the eluate recovered. For Day three oviduct samples, the half of the oviduct proximal to the fimbria was recovered by dissection and flushed with 0.5 ml of M2, while the portion of tract from mid-oviduct to mid-uterus was used for the collection of embryos from the utero-tubal junction by flushing. Preweighed squares (approximately 4 mm × 4 mm) of amino acid-free GF/C paper (Whatman, Clifton, NJ) were used to absorb uterine fluid from freshly dissected

Accepted July 15, 1986.
Received October 15, 1985.
[1] This research was supported by a Medical Research Council of Canada operating grant to G.A.S.
[2] Reprint requests.

uteri. Each square was placed gently on the uterine luminal surface at multiple sites, immediately reweighed, and placed in a 0.5-ml Eppendorf tube containing 50 μl of triple distilled water. The aqueous samples were then removed to other tubes, subjected to lyophilization and redissolved in 5 μl of triple distilled water.

Any samples that contained blood or tissue contamination were not used for analysis. To all sample preparations was added 12 μl of 10% trichloroacetic acid (TCA) containing 1 nmol of norleucine (as an internal standard). The suspensions were mixed gently and left on ice for 30 min. The TCA-soluble fraction was recovered after centrifugation for 10 min at 10,000 × *g*.

Amino Acid Analysis

The chromatography system was calibrated and all TCA-soluble fractions quantified as described previously (Schultz et al., 1981). All values were adjusted for manipulative losses by correcting relative to the internal norleucine standard.

Calculations and Statistical Procedures

The values in Table 2 were calculated by dividing the amino acid content of the absorbed fluid by its volume, assuming a specific gravity of 1.0. In Table 3, egg and morulae values were calculated by dividing amino acid content by a calculated spherical volume by using an egg and morula radius of 0.64 μm (Altman and Dittmer, 1962; Daniel, 1964). Blastocyst cavity fluid volume was directly measured. Blastocyst cellular volume was estimated by multiplying cavity volume by the ratio of trophectoderm weight to total blastocyst weight (Hafez and Sugawara, 1968). The percent composition of each amino acid was also calculated (enclosed in parentheses). Oviduct fluid volume measurements were not made so that only the percent composition of oviduct fluid is tabulated (Table 1).

Amino acid quantifications represent the mean and SEM-derived from amino acid analyses of six to eight samples for reproductive tract fluids, four samples for eggs and morulae (each sample contained from 150 to 200 eggs or morulae), and six samples for blastocysts. The smallest quantity of total amino acids measured in these experiments was about 500 pmol.

RESULTS

The amino acids threonine, glutamine, asparagine, and serine have similar mobilities and thus elute very closely. In embryo samples, except for the morula, there was enough separation to allow discrimination and quantification of threonine, glutamine-asparagine, and serine peaks. In oviduct and uterine samples, however, the glutamine-asparagine peak was enclosed by the overlapping threonine and serine peaks; thus, no accurate estimate could be made of its magnitude. It was, however, low, of the order of less than 2% of the total. This was in contrast to the high levels of glutamine-asparagine found in serum. Glutamine-asparagine comprised about 17% of the total in our determinations of amino acid content in rabbit serum (data not shown), compared to 15% in a previous study (Jaszczak et al., 1972) and 20% in mouse serum (Schultz et al., 1981). Tryptophan was not present at detectable levels in any sample. Cysteine is susceptible to oxidation and is not usually detected in our procedures. Proline, a secondary amine, cannot be detected by the o-phthalaldehyde method. Table 1 contains amino acid composition data of oviduct fluid. Amino acid concentration, composition, and composition excluding glycine for uterine fluid and embryos are shown in Tables 2 and 3 respectively. The pair of values enclosed in parentheses are composition and composition excluding glycine, respectively. Since glycine has such a great effect on both total concentration and composition, values

TABLE 1. Amino acid (A.A.) composition of oviduct fluid.

A.A.	Day 1 (% of Total)	Day 3 (% of Total)	Day 6 (% of Total)
TAU	7[a]	6	6 ± 1
ASP	2	1	1
THR	12	11	7
GLN and ASN			
SER	6	10 ± 1	4
GLU	9 ± 1	10 ± 1	8 ± 1
GLY	38 ± 3	33 ± 1	42 ± 1
ALA	13 ± 1	16 ± 1	18 ± 2
VAL	3	3	3
MET	1	2	1
ILE	1	1	1
LEU	2	2	2
TYR	1	1	1
PHE	1	1	1
HIS	1	2	3
LYS	3	2	2
ARG	2	2	2

[a]SEM are not shown when they are below 0.5%. The SEM are generally less than 0.1% for those amino acid composition values of from 1 to 3.

TABLE 2. Amino acid (A.A) concentration and composition in uterine fluid.

A.A.	Day 1 (mM)	Day 3 (mM)	Day 6 (mM)
TAU	3.41 ± 0.53(18,23)[a]	1.37 ± 0.05(5,14)	1.38 ± 0.03(5,21)
ASP	0.28 ± 0.06(2,3)	0.27 ± 0.06(1,3)	0.14 ± 0.03(1,4)
THR	1.85 ± 0.08(10,13)	0.55 ± 0.07(2,5)	0.56 ± 0.04(<1,3)
GLN and ASN[b]			
SER	1.09 ± 0.05(6,8)	1.79 ± 0.16(6,17)	0.80 ± 0.10(3,13)
GLU	3.36 ± 0.24(18,23)	3.46 ± 0.45(12,33)	0.59 ± 0.04(2,8)
GLY	4.15 ± 0.14(22,–)	18.44 ± 0.57(64,–)	19.33 ± 0.69(76,–)
ALA	2.18 ± 0.14(12,15)	1.09 ± 0.12(4,11)	1.24 ± 0.24(5,21)
VAL	0.33 ± 0.02(2,2)	0.27 ± 0.02(1,3)	0.18 ± 0.04(1,4)
MET	0.32 ± 0.04(2,2)	0.23 ± 0.12(<1.2)	0.10 ± 0.00(<1,2)
ILE	0.21 ± 0.02(1,1)	0.08 ± 0.00(<1,1)	0.11 ± 0.01(<1,2)
LEU	0.49 ± 0.06(3,3)	0.19 ± 0.01(1,2)	0.20 ± 0.02(1,3)
TYR	0.12 ± 0.05(1,1)	0.10 ± 0.00(<1,1)	0.09 ± 0.00(<1,1)
PHE	0.11 ± 0.01(1,1)	0.09 ± 0.01(<1)	0.08 ± 0.00(<1,1)
HIS	0.23 ± 0.02(1,2)	0.14 ± 0.01(1,1)	0.34 ± 0.08(1,5)
LYS	0.26 ± 0.02(1,2)	0.18 ± 0.02(1,2)	0.23 ± 0.04(1,4)
ARG	0.30 ± 0.01(2,3)	0.18 ± 0.00(1,2)	0.17 ± 0.00(1,3)
Total	18.69 ± 1.50	28.75 ± 1.68	25.54 ± 1.36
Total minus GLY	14.54	10.31	6.21

[a]The first number in parentheses is the percentage that the amino acid represents of the total amino acid content measured; the second number represents the percentage calculated when glycine is excluded (see text).

[b]The glutamine-asparagine peak was not well resolved and could not be accurately quantified. The combined value is low (<2% of the total, see text).

TABLE 3. Amino acid (A.A) concentration and composition in rabbit embryos.

A.A.	Egg (mM)	Morulae (mM)	Blastocyst cavity (mM)	Blastocyst cellular (mM)
TAU	7.58 ± 0.48(37,42)[a]	2.12 ± 0.14(7,10)	0.09 ± 0.02(1,4)	<0.02
ASP	2.97 ± 0.52(15,17)	4.78 ± 0.20(15,21)	0.02 ± 0.00(<1,3)	0.20 ± 0.03(3,4)
THR	0.17 ± 0.02(1,1)	0.77 ± 0.12(2,3)	0.39 ± 0.01(3,9)	0.24 ± 0.01(4,6)
GLN and ASN[b]	0.20 ± 0.02(1,1)		0.21 ± 0.02(2,6)	0.11 ± 0.01(2,3)
SER	0.39 ± 0.03(2,2)	1.92 ± 0.30(6,9)	0.77 ± 0.06(6,18)	0.37 ± 0.02(6,9)
GLU	2.71 ± 0.26(13,15)	5.08 ± 0.58(16,23)	0.14 ± 0.02(1,3)	1.30 ± 0.08(22,31)
GLY	2.56 ± 0.14(13,–)	9.57 ± 0.55(30,–)	8.14 ± 0.41(67,–)	1.71 ± 0.24(30,–)
ALA	2.06 ± 0.20(10,11)	2.32 ± 0.32(7,10)	1.06 ± 0.23(9,27)	0.58 ± 0.04(10,14)
VAL	0.31 ± 0.04(2,2)	0.63 ± 0.04(2,3)	0.14 ± 0.01(1,3)	0.19 ± 0.02(3,4)
MET	0.11 ± 0.02(1,1)	0.68 ± 0.02(2,3)	0.07 ± 0.00(1,3)	0.13 ± 0.02(1,1)
ILE	0.15 ± 0.02(1,1)	0.17 ± 0.02(1,1)	0.06 ± 0.00(1,3)	0.12 ± 0.01(2,3)
LEU	0.37 ± 0.04(2,2)	0.63 ± 0.09(2,3)	0.10 ± 0.00(1,3)	0.24 ± 0.02(4,6)
TYR	0.07 ± 0.01(<1,1)	0.23 ± 0.02(1,1)	0.07 ± 0.00(1,3)	0.10 ± 0.01(2,3)
PHE	0.14 ± 0.02(1,1)	0.29 ± 0.04(1,1)	0.05 ± 0.00(<1,2)	0.11 ± 0.01(2,3)
HIS	0.22 ± 0.05(1,1)	0.32 ± 0.07(1,1)	0.09 ± 0.01(1,3)	0.13 ± 0.02(2,3)
LYS	0.11 ± 0.00(1,1)	1.04 ± 0.07(3,4)	0.51 ± 0.04(4,12)	0.26 ± 0.02(4,6)
ARG	0.20 ± 0.02(1,1)	1.08 ± 0.06(3,4)	0.22 ± 0.03(2,6)	0.15 ± 0.04(3,4)
Total	20.32 ± 1.90	31.63 ± 2.62	12.14 ± 0.76	5.84 ± 0.60
Total minus GLY	17.76	22.06	4.00	4.13

[a]The first number in parentheses is the percentage that the amino acid represents of the total amino acid content measured; the second number represents the percentage calculated when glycine is excluded (see text).

[b]The glutamine-asparagine peak was not well resolved and could not be accurately quantified. The combined value is low (<2% of the total, see text).

ignoring the contribution of glycine have been tabulated to aid interpretation.

While the amino acid composition of the oviduct was observed to be essentially invariant over the preimplantation period, both amino acid concentration and composition of embryos and uterine fluids changed during this interval. The total amino acid concentration of the uterus increased with development (solely due to the contribution of glycine), while in the embryo, total amino acid concentration reached its zenith at the morula stage and declined in both compartments of the blastocyst. Inspection of Tables 1, 2, and 3 reveals that a developmental change in composition will not necessarily predict the character of the change in concentration for an individual amino acid. The developmental profile or amino acid content is complex, but some general trends can be discerned. Glycine increased in reproductive tract fluids and embryos (except the blastocyst cellular compartment) with development. Taurine, initially high, subsequently decreased in amount in both embryos and reproductive tract fluids. The neutral amino acids leucine, valine, phenylalanine, and methionine were almost uniformly low in both embryos and reprodictuve tract fluids and showed little developmental modulation. Aspartic acid, serine, glutamic acid, and alanine were more prevalant and more likely to differ between developmental stages and between reproductive tract fluids and embryos. Changes in amino acid composition with development were somewhat less marked when glycine was excluded from the calculations.

DISCUSSION

Glycine has a great effect on the total amino acid concentration in reproductive tract fluids and embryos. The total amino acid concentration in uterine fluids increases with development, but the total excluding glycine actually decreases significantly. The differences in total amino acid concentrations between egg and morula and between blastocyst cavity and cellular compartments are attributable to differences in glycine. In addition, percent composition calculated with glycine presents a developmental pattern of change different from that when glycine is excluded from the calculation of composition. This is especially notable with taurine, threonine, serine, glutamic acid, and alanine. The prominence of glycine has been reported in previous studies involving Day seven rabbit embryos (Jaszczak et al., 1972), preimplantation mouse development (Schultz et al., 1981) and early sea urchin development (Fry and Gross, 1970). Glycine may have a unique role in development. In the mouse, however, the high glycine content in the egg decreases dramatically by the blastocyst stage (Schultz et al., 1981). This corresponds developmentally to an increased capacity to transport glycine as well as to an increased utilization of glycine in intermediary metabolism and macromolecular synthesis (Hobbs and Kaye, 1985).

Taurine, present at high levels in the reproductive tract fluids and embryos of early rabbit preimplantation development (and in the mouse, Schultz et al., 1981), possibly has a role in capacitation and fertilization (Mrsny et al., 1979; Meizel et al., 1980) and in sperm motility (Leibfreid and Bavister, 1981). The neutral and basic amino acids, although representing only a small fraction of the total, are present in concentrations greater than those required for optimal growth in embryo culture (Daniel and Krishnan, 1967; Daniel and Olsen, 1968; Mauer et al., 1968). Arginine and lysine are present in higher concentrations than the neutral amino acids, and this may be important to the synthesis of the histones as cell proliferation accelerates.

Although there is a rough correspondence between pools of reproductive tract fluids and embryos, concentration gradients exist at all stages, and involve levels of all amino acids. It is evident that preimplantation embryos can establish autonomy from their uterine environment. There is a substantial reverse gradient of glycine between the morula and Day three uterine pools. At Day six, large gradients in glycine concentration exist between uterine fluid, the blastocyst cavity, and the blastocyst cellular compartment. The gradients of most other amino acids (and of the total amino acid concentration minus glycine), however, are more modest.

The cavity pool may be particularly significant in terms of amino acid dynamics. The presence of amino acids in the cavity is compatible with the high level of transport through the trophectoderm into the cavity previously reported (Miller and Schultz, 1983). Evidence for amino acid transport systems on the inner face of the trophectoderm (Miller and Schultz, 1983) suggests the possibility that the cavity could act as an amino acid reservoir. This is compatible with the report that an increase in protein content of the blastocyst cavity over the developmental period from seven days to nine days was accompanied by a

significant reduction in cavity content of alpha-amino nitrogen (Lesinski et al., 1967).

ACKNOWLEDGMENTS

We are grateful to Dr. D. Mckay for developing the elution system used in this study and for operation of the amino acid analyzer.

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