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The erythrocyte cytoskeleton protein 4.2 is not demonstrable in several mammalian species

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Abstract

Erythrocyte membrane proteins from 44 representative mammals were studied. Protein 4.2 was not detected in guinea pigs (*Cavia porcellus*) (N = 14), Southern Brazilian swamp large rats (*Myocastor coypus*) (N = 2), cutias (*Dasyprocta* sp) (N = 4), and horses (*Equus caballus*) (N = 13). These animals also presented high ankyrin concentrations except for the horse which did not exhibit a sharp band, although minor components located between proteins 2 and 3 could account for the ankyrin family. The rodents studied did present band 6, which was not detectable in other common rodents such as white rats (*Rattus norvegicus*) (N = 9) and mice (*Mus musculus*) (N = 12). Since the absence of protein 4.2 does not disrupt the cytoskeleton membrane, we suggest that it is not an essential protein. Its absence may be compensated physiologically by the higher ankyrin concentration observed.

Key words
- Protein 4.2
- Erythrocyte membrane cytoskeleton
- Mammals

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Human erythrocyte membrane proteins have been extensively studied, and band 3 and glycophorins have been classified as integral proteins, and the spectrins, band 2.1, band 4.1, band 4.2, band 4.5, band 4.9, band 5 (actin), band 6 (glyceraldehyde-3-phosphate dehydrogenase), and band 7 as peripheral proteins. The peripheral proteins which form the cytoskeleton are very important for maintaining the protein network just below the membrane lipid bilayer (1). In general, mammals present small variations in erythrocyte membrane protein concentration as well as in molecular weight (2). Variable spectrin and ankyrin concentrations have been reported in mice (3-8), as well as a molecular weight variation of band 3 in non-human primates (9), its enrichment in llamas (10), and the absence of band 6 in rats and mice (11,12).

In the course of an extensive investigation of red cell membrane proteins (2), 44 selected representatives of mammalian species belonging to 13 orders from Brazilian Zoos and Brazilian Research Institutes were investigated (Table 1). Red cells were collected into acid-citrate-dextrose solution, washed in buffered saline and lysed in cold 5

Table 1 - Mammals whose erythrocyte membrane proteins were studied and their relative amount of protein 4.2.

*The densitometer was not able to discriminate between band 4.1 and band 4.2, although both bands could be observed visually.

Scientific name	N	Family	Order	band 4.2 percent (SD)
Homo sapiens	20	Hominidae	Primates	7.5 (1.7)
Cebus apella	15	Cebidae	Primates	9.4 (1.5)
Alouatta sp	6	Cebidae	Primates	8.0 (2.2)
Ateles paniscus chamek	4	Cebidae	Primates	6.5 (1.2)
Gorilla gorilla	1	Pongidae	Primates	7.1
Pongo pygmaeus	2	Pongidae	Primates	5.4 (1.0)
Erythrocebus pata	2	Cercopithecidae	Primates	9.5 (1.2)
Papio cynocephalus	1	Cercopithecidae	Primates	5.9
Arctocephalus tropicalis	2	Otariidae	Pinnipedia	7.7 (0.7)
Arctocephalus australis	1	Otariidae	Pinnipedia	7.2
Acinonyx jubatus	1	Felidae	Carnivora	5.8
Felis concolor	1	Felidae	Carnivora	7.3
Panthera onca	3	Felidae	Carnivora	8.9 (1.0)
Panthera leo	6	Felidae	Carnivora	8.9 (1.8)
Panthera tigris	2	Felidae	Carnivora	8.0 (2.2)
Panthera pardus	2	Felidae	Carnivora	7.0 (1.5)
Procyon cancrivorus	1	Procyonidae	Carnivora	4.3
Nasua nasua	6	Procyonidae	Carnivora	8.1 (0.6)
Chrysocyon brachyurus	6	Canidae	Carnivora	6.7 (0.4)
Cerdocyon thous	6	Canidae	Carnivora	8.6 (1.8)
Canis familiaris	13	Canidae	Carnivora	7.0 (1.3)
Cavia porcellus	14	Caviidae	Rodentia	0
Rattus novergicus	9	Muridae	Rodentia	10.2* (0.6)
Mus musculus	12	Muridae	Rodentia	5.9 (1.3)
Myocastor coypus	2	Myocastoridae	Rodentia	0
Dasyprocta sp	4	Dasyproctidae	Rodentia	0
Mesocricetus auratus	8	Cricetidae	Rodentia	12.9* (0.9)
Oryctolagus cuniculus	15	Leporidae	Lagomorpha	7.4* (1.2)
Bradypus tridactylus	2	Bradypodidae	Edentata	10.2* (0.9)
Elephas maximus	1	Elephantidae	Proboscidea	5.1
Camelus bactrianus	3	Camelidae	Artiodactyla	6.3 (0.9)
Giraffa camelopardalis	1	Giraffidae	Artiodactyla	5.2
Cervus elaphus	2	Cervidae	Artiodactyla	6.0 (0.7)
Ozotoceros bezoarticus	1	Cervidae	Artiodactyla	5.7
Mazama gouazoubira	3	Cervidae	Artiodactyla	7.5 (1.4)
Ovis aries	15	Bovidae	Artiodactyla	5.4 (1.1)
Bos taurus	6	Bovidae	Artiodactyla	10.1* (1.1)
Bos indicus	15	Bovidae	Artiodactyla	10.6* (1.9)
Capra hircus	19	Bovidae	Artiodactyla	3.2 (0.2)
Tapirus terrestres	1	Tapiridae	Perissodactyla	12.3*
Equus callus	13	Equidae	Perissodactyla	0
Inia geoffrensis	6	Iniidae	Cetacea	10.9* (0.9)
Tadarida brasiliensis	1	Molossidae	Chiroptera	9.5*
Trichechus inunguis	4	Trichechidae	Sirenia	11.1 (1.7)
Didelphis marsupialis	8	Didelphidae	Didelphimorphia	8.8 (1.7)

mM sodium phosphate buffer, pH 8.0, containing the following protease inhibitors: 0.2 mM phenylmethylsulfonyl fluoride, 0.2 mM N-ethylmaleimide, 1.0 mM disodium ethylenediaminetetraacetate, 0.1 mM diisopropylfluorophosphate, 0.1 mM Na-p-Tosyl-L-lysine chloromethyl ketone, 0.1 mM *p*-hydroxymercuribenzoic acid, and 1 µM pepstatin A.

The ghosts were washed with the same buffer, solubilized by standard methods and submitted to SDS-polyacrylamide gel electrophoresis (SDS-PAGE), using 10% polyacrylamide gel (13) as well as an exponential gradient (3-17%, with piston) polyacrylamide gel (14). A ghost sample without protease inhibitors was also prepared.

Coomassie Blue (0.605 mM) in isopropanol:acetic acid:water (5:2:6) was used to stain the SDS gels. An Ultrascan XL laser densitometer (Pharmacia) was used with gelscan/92 software. Figure 1 illustrates the SDS-PAGE of the erythrocyte proteins from the animals which did not present protein 4.2 and shows that the patterns were constant in the presence of individual inhibitors and the pool of all inhibitors.

The red cell morphology of all animals lacking band 4.2 is shown in Figure 2. In comparison with human red cells, it can be seen that *Equus caballus* erythrocytes are microcytic and that Rodentia erythrocytes are similar to human except for those of *Dasyprocta* sp, which exhibit occasional stomatocytes. However, it is difficult to ascribe these morphological differences to the absence of protein 4.2.

The following Rodentia representatives did not contain demonstrable protein 4.2: guinea pig (*Cavia porcellus*) (N = 14), Southern Brazilian swamp large rats (*Myocastor coypus*) (N = 2), and cutias (*Dasyprocta* sp) (N = 4). Horses (*Equus caballus*) (N = 13) also did not exhibit protein 4.2. These animals also presented high ankyrin concentrations except for horses, which did not exhibit a sharp band although they showed a group

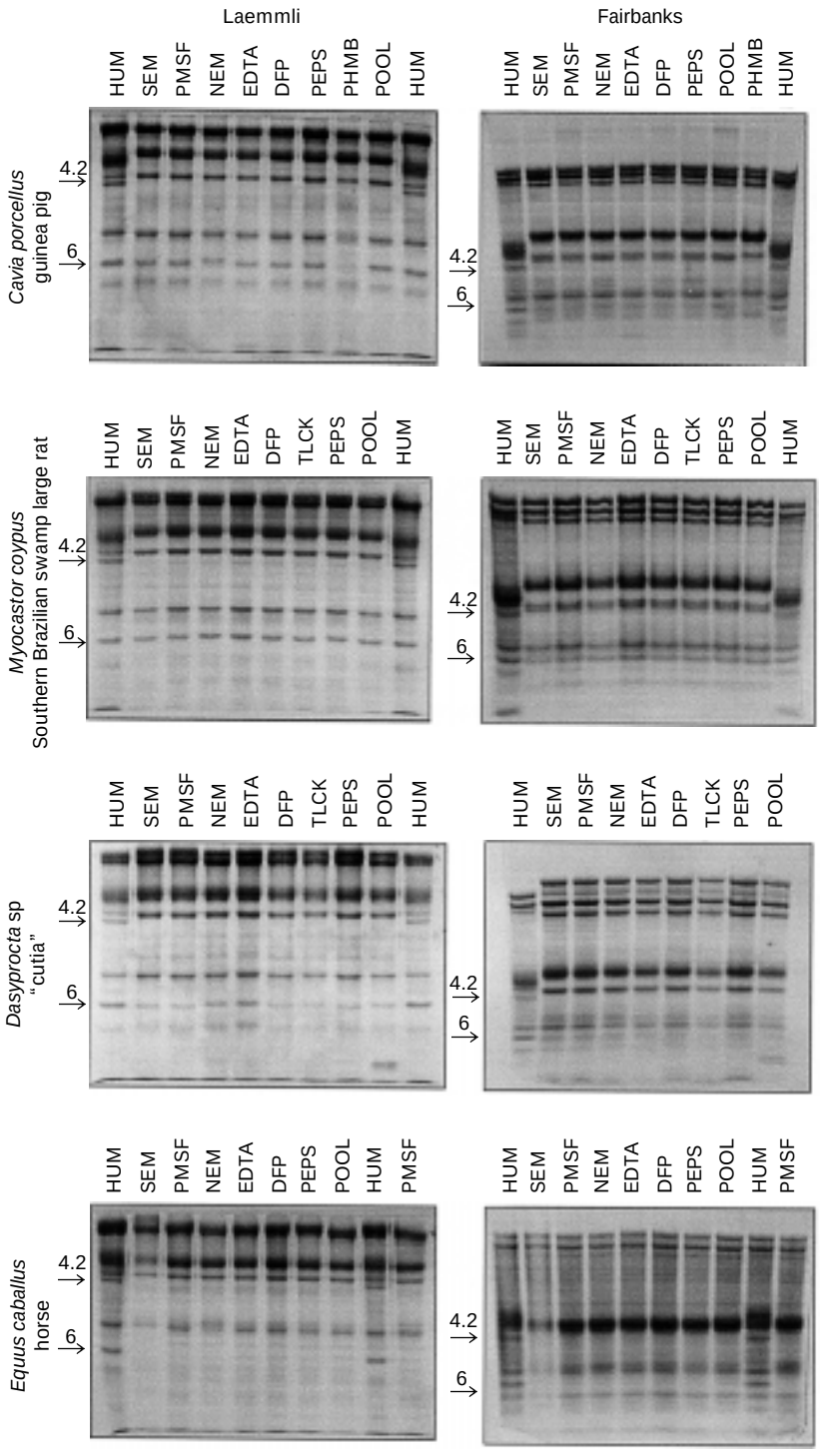


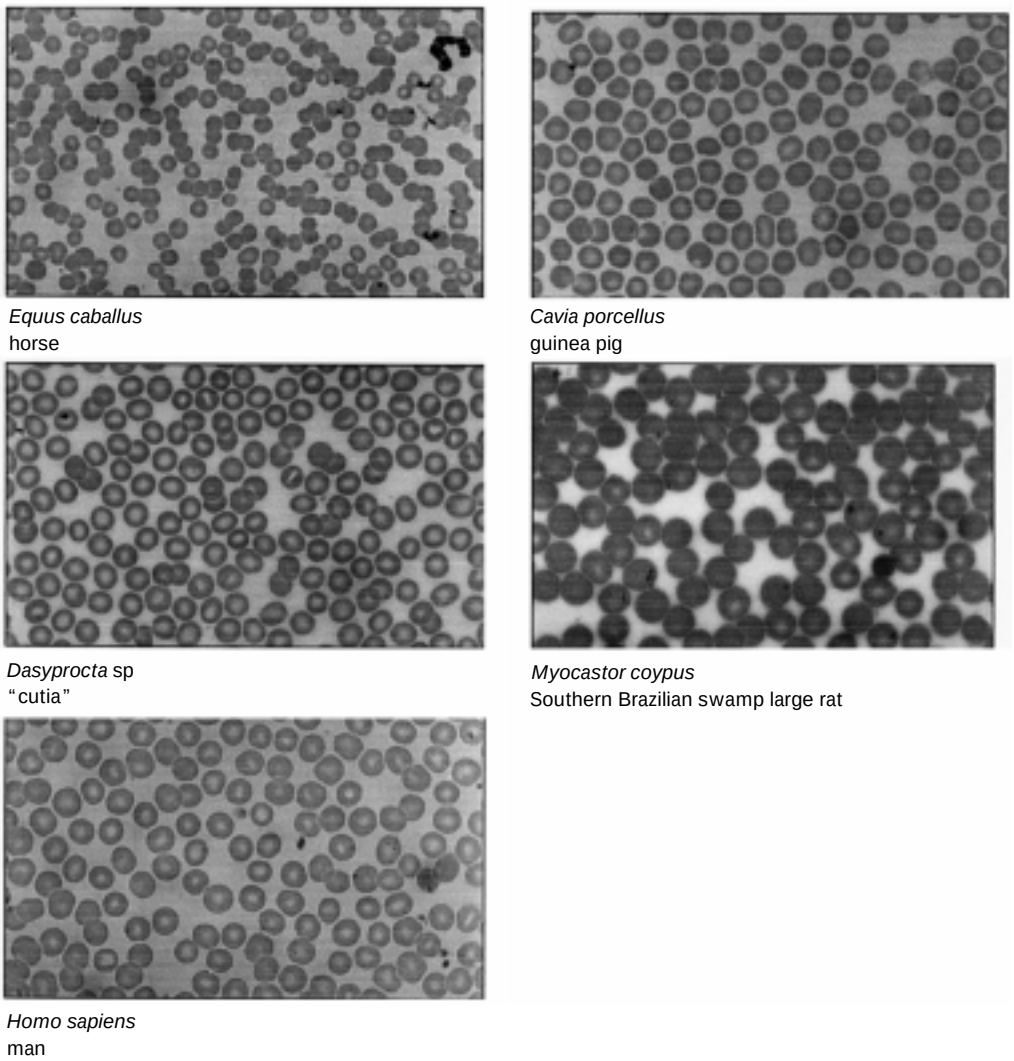
Figure 1 - Absence of erythrocyte membrane protein 4.2 in *Cavia porcellus*, *Myocastor coypus*, *Dasyprocta* sp and *Equus caballus* in SDS-PAGE. HUM, Human erythrocyte membrane protein; SEM, without any inhibitor; PMSF, phenylmethylsulfonyl fluoride; NEM, N-ethylmaleimide; EDTA, disodium ethylenediaminetetraacetate; DFP, diisopropylfluorophosphate; PEPS, pepstatin A (isovaleryl-VaL-VaL--Sta-Ala-Sta); PHMB, *p*-hydroxymercuribenzoic acid; POOL, mixture of all inhibitors; TLCK, Na-p-Tosyl-L-lysine chloromethyl ketone.

of minor components located between proteins 2 and 3, which could account for an ankyrin family. Protein 4.2 has been considered to play an important role in cytoskeleton anchorage to the integral protein 3, together with ankyrin (band 2.1).

Inaba and Maede (15) reported the finding of a putative membrane protein 4.2 between 4.1a and 4.1b of horse erythrocyte membrane. This, however, was not observed in our study. Bands 4.1a and 4.1b always remain together, and the hypothesis raised by these investigators of an undefined band between 4.1a and 4.1b may be due to an artifact since an antibody against 4.2 was not employed in their study. If a band 4.2 exists in horse erythrocytes, it should be detected as a protein of lower molecular weight than proteins 4.1a and 4.1b.

The rodent species studied here did present band 6, which does not occur in other common Rodentia (11,12) such as white rats (*Rattus norvegicus*) (N = 9) and mice (*Mus musculus*) (N = 12) (see Figure 1).

Protease inhibitors were employed in order to exclude the possibility that the absence of band 4.2 was due to proteolysis. However, since antibodies to band 4.2 were



Equus caballus
horse

Cavia porcellus
guinea pig

Dasyprocta sp
"cutia"

Myocastor coypus
Southern Brazilian swamp large rat

Homo sapiens
man

Figure 2 - Red cell morphology of animals lacking protein 4.2. Human erythrocytes are used as a comparative sample. Magnification: 1000X.

not used in the present study, the possibility of a band immunologically similar to the protein of band 4.2 but of different molecular weight cannot be ruled out.

Our data, however, do suggest that protein 4.2 is not an essential protein since its absence does not disrupt the cytoskeleton membrane. Physiologically its absence may be compensated for by the higher ankyrin concentration observed in the mammals lacking band 4.2.

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