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FISH EYE-LENS REAGENTS: SEX-SPECIFIC AGGLUTINATION OF HUMAN ERYTHROCYTES

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A possible new class of reagents, prepared in vitro from fish eye lenses, was reported recently to have potential application in the identification of selected molecules and cells (1). These reagents, by differential precipitation or agglutination reactions, showed induced specific affinities for hemoglobins A and S, for human and bovine serum albumins, and for human erythrocytes belonging to blood groups A_1, A_2, B, and O.

Objectives of the present study were to (a) test further the specificity and resolving power of lens reagents by attempting to detect differences between erythrocytes of human females and males (2), (b) determine if there is any correlation between Rh type or ABO blood group and reactivity of lens reagents, and (c) provide initial quantitative information on lens reagents.

MATERIALS AND METHODS

LENS COLLECTION AND EXTRACTION.—Lenses were collected from eight skipjack tuna (*Katsuwonus pelamis* L.), the same species as used in the initial lens reagent study. The fish had the following fork lengths (cm): 41, 46, 47, 48, 50, 51, 52, and 55. All had been caught on November 10, 1983, near Cedros Island, Baja California, Mexico, and stored on ice until the lenses were removed. After storage in dry, sealed containers at −10° for approximately 6 months, the lenses were further processed according to a method modified slightly from that previously reported (1) and outlined as follows. Lens cores (nuclei) were obtained by dissection and ground with mortar and pestle. The lens particles were extracted at ambient temperature (23°) with a volume of weak saline solution (0.018 g/dl NaCl) equal to twice that of the saline-saturated lens tissue. After 24 h, the mixture was centrifuged at 10,000 rpm for 4 min at ambient temperature.

PREPARATION OF COLD PRECIPITABLE PROTEIN (CPP) SOLUTION.—The supernatant was refrigerated (5°) for 4 days which yielded a large precipitate of CPP. The supernatant was decanted, and the precipitate was washed with cold distilled H_2O and then dissolved at ambient temperature in a volume of normal saline solution (0.9 g/dl NaCl) equal to ten times the volume of the precipitate.

PREPARATION OF ERYTHROCYTE SAMPLES.—Human erythrocytes were obtained from anticoagulated EDTA, [Ethylenediaminetetraacetic acid (tripotassium salt)] blood samples taken from 20 females and 20 males, all randomly selected. The blood samples were washed three times with normal saline solution and then tested as to Rh type (+ or −) and ABO group (A, B, O, or AB), using commercial antisera (Ortho Diagnostic Systems Inc., Raritan, New Jersey). Testing methods were those recommended by the American Association of Blood Banks (3). In addition, equal portions of blood from each tube were mixed to form separate pools of erythrocytes from females and males.

MODIFICATION OF CPP SOLUTION FOR SEX-SPECIFIC REACTIVITY WITH ERYTHROCYTES.—Equal volumes of CPP solution were mixed at ambient temperature with equal volumes of a 3% suspension (in normal saline solution) of pooled, packed erythrocytes from females or males. The CPP/erythrocyte mixtures were warmed in a water bath at 40° for 4 min, refrigerated for 24 h, rewarmed until the CPP precipitate dissolved, and then centrifuged at 3,400 rpm for 1 min at ambient temperature. The supernatants were decanted from the cell pellets and as before, erythrocytes (from the same sex) were added and the suspensions were warmed, refrigerated, rewarmed, and centrifuged. The final supernantants were diluted with 2 volumes of normal saline solution.

Samples of each erythrocyte-treated CPP solution were absorbed to remove possible cross-reactivity and also to make negative absorbed controls. These absorptions were achieved by gently mixing separate CPP samples at ambient temperature for 15 min with equal volumes of pooled,

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packed erythrocytes from females or males. The mixtures were centrifuged at 3,400 rpm for 1 min at ambient temperature, and the supernatants were removed. This procedure was repeated four times, using fresh cells from the same (female or male) pool each time. Subsequent studies were carried out in duplicate.

AGGLUTINATION TESTS.—*Preliminary Study with Pooled Blood* (format as in Table 1).—Control.—One drop of unmodified CPP solution, either unabsorbed or absorbed, was separately mixed in a 10×75 mm test tube with 4 drops of a 3% suspension of pooled erythrocytes from females or males and 4 drops of normal saline solution. Experimental—Same as in the control group except that erythrocyte-modified CPP solution was substituted for unmodified CPP solution. All tubes were centrifuged at 3,400 rpm for 1 min at ambient temperature and examined for signs of agglutination.

TABLE 1. Agglutination Reactions of Pooled Erythrocytes

Lens extract	Erythrocytes	
	Female	Male
Unmodified CPP (control)		
unabsorbed	2+[a]	2+
absorbed with erythrocytes from		
females	w+	w+
males	w+	w+
CPP solution modified with erythrocytes from females		
unabsorbed	4+	4+
absorbed with erythrocytes from		
females	w+	w+
males	2+	w+
CPP solution modified with erythrocytes from males		
unabsorbed	4+	4+
absorbed with erythrocytes from		
females	w+	2−
males	w+	w+

[a]Reactions are graded 0 (no agglutination), w+ (weakly positive, trace), or 1-4+ (4+=maximal agglutination) (3).

Individual Blood Study (format as in Table 2).—Modified/absorbed CPP solutions that differentially agglutinated the pooled erythrocytes from one or the other sex were tested as above for reactivity with erythrocytes from individual females and males. These CPP solutions will henceforth be referred to as female and male lens reagents.

QUANTITATIVE STUDIES.—Potency and protein concentrations of female and male lens reagents were determined by agglutination testing after serial dilutions (0-1:18) with normal saline solution and by a standard colorimetric protein assay (4), respectively. Additionally, a control solution of bovine serum albumin (BSA) (Grade 5, Sigma Chemical Co., St. Louis, Missouri) was prepared in normal saline at the same concentration (4.20 mg/ml) as lens reagents, and mixed with pooled erythrocytes from females or males to test for nonspecific agglutination.

RESULTS

AGGLUTINATION TESTS.—*Preliminary Study* (Table 1).—Unmodified CPP solution (control) showed medium (2+) reactivity with erythrocytes from both sexes. After absorption with erythrocytes from either females or males, this nonspecific reactivity decreased considerably. CPP solutions modified with erythrocytes from females or males showed increased (4+) nonspecific reactivity. However, these erythrocyte-modified CPP solutions, after absorption with erythrocytes from the opposite sex, lost most of their reactivity for eryththrocytes of the opposite sex, but now showed medium reactivity for erythrocytes from the same sex (and constitute female or male lens reagents). Absorption with erythrocytes of the same sex as used to modify the CPP resulted in loss of most reactivity for erythrocytes from both sexes.

Individual Study (Table 2).—The

TABLE 2. Blood Types, Groups, and Agglutination Reactions of Blood Samples from Females and Males

Blood samples	Rh	ABO	Agglutination reaction with	
			Female lens reagent	Male lens reagent
No. females				
8	+	A	2+[a]	w+
1	−	A	2+	w+
5	+	B	2+	w+
10	+	O	2+	w+
1	−	O	2+	w+
2	+	AB	2+	w+
No. males				
4	+	A	w+/0 (1 individual)	2+
1	−	A	w+	2+
6	+	B	w+	2+
1	−	B	w+	2+
10	+	O	w+/0 (3 individuals)	2+
1	−	O	w+	2+
4	+	AB	w+	2+

[a]Reactions are graded as in Table 1.

blood samples from females and males were predominantly Rh+ (only 2 females and 3 males were Rh−) and belonged to all major groups in the ABO system.

Female and male lens reagents gave medium reactions with all erythrocyte samples from the corresponding sex. Trace or O reactions (the latter in 4 males) occurred with erythrocytes from the opposite sex.

Quantitative Studies (Table 3).—Female and male lens reagents showed a range of potency (2+ −w+) at different dilutions (0-1:12) and protein concentrations (4.20-0.31 or 0.32 mg/ml). No agglutination of erythrocytes from either sex occurred in the BSA solution.

DISCUSSION

The reactions of unmodified CPP (control), both unabsorbed and absorbed, confirmed the findings from previous studies (1) that CPP tends to bind nonspecifically to erythrocytes, as well as to molecules in solution. Reactivity was strengthened by the contact/separation treatment of CPP with erythrocytes

TABLE 3. Dilutional Changes in Potency and Concentration of Female and Male Reagents

Reagent (female or male) dilution	Agglutination reaction with erythrocytes from corresponding sex	Reagent concentration (mg/ml)	
		by dilution	by Bradford method
0	2+[a]	4.20	4.20
1:1	2+	2.10	2.10
1:2	1+	1.40	1.40
1:4	1+	0.84	0.82
1:6	1+	0.60	0.56
1:8	1+	0.47	0.46
1:10	w+	0.38	0.38
1:12	w+	0.32	0.31
1:14	0	0.28	0.27
1:16	0	0.25	0.24
1:18	0	0.22	0.21

[a]Reactions are graded as in Tables 1 and 2.

from females or males. This procedure also appeared to induce formation of a subpopulation of proteins that reacted specifically with pooled erythrocytes from one or the other sex. These proteins were demonstrated after removal by absorption of nonspecifically reacting proteins.

The tests with blood samples from individual females or males confirmed the sex-specific reactivity of the lens reagents. There was no apparent relationship between blood type or group and reaction with lens reagent.

The quantitative studies revealed that lens reagents are reactive over a broad range of dilutions and concentrations. Their reactivity can be strengthened to maximal (4+) by concentration (personal observation).

The absence of agglutination by erythrocytes from either females or males in BSA at a concentration of 4.20 mg/ml, the same as that of protein in lens reagents, indicates that protein concentration is not a factor in the sex-discriminating reactions of these reagents. The mechanism of action of lens reagents is not known, but it is suspected to be related to newly induced conformations (from previous exposure to "template" cells or molecules) that provide for a better fit in subsequent binding reactions (1).

The possible presence of sex-specific components on human erythrocytes was first suggested in a preliminary study using natural agglutinins from marine invertebrates and fishes (2). That such components exist now seems confirmed by the present study with lens reagents. From a comparative standpoint, it is of interest that sex-specific differences have also been demonstrated on the erythrocytes of fishes (5).

The ready availability of lens reagents may give cell biologists a new tool for elucidating cell surface structures relating to the sex, and possibly other characteristics, of individuals.

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